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# SYMPOSIUM ON THEORETICAL AND PRACTICAL ASPECTS OF POLYPLOIDY IN CROP PLANTS[1]

## INTRODUCTION

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THE purpose of this symposium is to evaluate recent progress in the field of induced polyploidy.

It is evident from the reports that induced polyploidy as a tool in plant breeding will soon be included in the text-books along with the classical methods of inbreeding and outbreeding.

In the subject-matter of the symposium the integrity of the chromosome is primarily maintained in contrast to the newly acquired concept of an artificially rebalanced genome. The latter is remolded in many different ways by doubling, quadrupling and trebling both pure and hybrid lines by using the spontaneously acquired parthenogenetic halved polyploids, by working from triploids down toward simpler polysomics and by stabilizing desirable types through redoubling of triploids, aneuploids and polysomics. Each intermediate step may render types of practical importance.

While this is being done to genomes leaving individual chromosomes intact, I venture to predict, on the basis of

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cytological observation, that chemicals will soon be used in order to interfere with the integrity of chromosomes. These will cause duplications, deletions and perhaps translocations, thus competing with x-rays and ultraviolet light which induce somatic mutations. Such chemicals may be applied at any given level of polyploidy.

The basic principles of polyploidy were well known before general methods of inducing this condition were developed. Thus with the advent of methods to induce polyploidy workers were prepared to use the new process advantageously. The historical and causal sequence of these events illustrates the fictitious nature of the man-made distinction between theoretical and applied work. The present status of development in this field should encourage workers in both theoretical and applied lines of research.

# POLYPLOIDY IN NICOTIANA

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## INTRODUCTION

THERE are about fifty natural species of Nicotiana. Most of them occur in subtropical and temperate regions of North and South America; the rest, a distinct group, are found in Australia and a few outlying islands. The greatest number of species occurs in South America, and they belong mostly to the 12-chromosome category; except for a small group of 9- and 10-chromosome species and the two cultivated 24-chromosome species, *N. rustica* and *N. Tabacum*. In contrast, the relatively few North American species are mostly of the 24-chromosome, but some of the 12-chromosome group. Members of the Australian group are not only unique as regards morphological features, but also as to chromosome numbers, which begin at 16 and present an unbroken series of even numbers up to 24, with a single polyploid 32-chromosome species lying beyond the range (Wheeler, 1935).

If we consider the American species, they may be classified as monobasic, those which have 12 (or rarely 9 or 10) chromosomes; or dibasic, those with 24 chromosomes. These latter, obviously polyploid species, may conceivably have risen in two different ways; either by doubling the chromosome number in some monobasic species, autopolyploidy, in which case the two sets of 12 chromosomes now present in them will have been derived from the same source; or by doubling of chromosome number in an interspecific hybrid of two monobasic species, amphidiploidy or allopolyploidy, in which case the two sets of chromosomes are derived from a different source.

There is abundant evidence that the two cultivated 24-chromosome species, *rustica* and *Tabacum*, are amphidiploid, and the relationship to specific 12-chromosome South American species has been well established. Thus

*Tabacum* appears to have arisen from hybridization of *sylvestris* and some member of the *tomentosa* assemblage followed by doubling of chromosome number; and *rustica* by a similar process from *paniculata* and *undulata* allies. The other North American 24-chromosome species also appear to be amphidiploid, but their origin has not been so satisfactorily established (Goodspeed, 1934). On the most conservative basis, there are at least three distinct 24-chromosome North American species and there seem to be only two distinct, indigenous 12-chromosome species in this same region. In these circumstances it is obviously difficult to account for all the 24-chromosome species by amphidiploidy.

Our studies of polyploidy in Nicotiana have not been concerned so much with that phenomenon *per se,* as with its relation to problems of the origin of the polyploid species, particularly that of the cultivated species, *Tabacum.* As a consequence our experimental efforts to induce polyploidy have been restricted until recently to those species and hybrids which might have some bearing upon that problem. As that problem has been pursued, numerous ramifications have been disclosed which require further investigation; among them one which appears to be of considerable importance and worthy of investigation despite its difficulty, namely, that of the nature and character of the genetic alterations which have occurred in the transformation of what may be called a raw amphidiploid, such as amphidiploid *sylvestris-tomentosa,* into a finished product, such as the present equivalent species, *Tabacum.*

### AUTOPOLYPLOIDY

Autopolyploidy may now readily be induced experimentally in Nicotiana. By the use of heteroauxin on the cut surface of decapitated stems as described by Greenleaf (1938) we have induced callus growth and the subsequent production of polyploid callus shoots in a number of species; among them *Tabacum, sylvestris, tomentosi-*

*formis,* a number of different selections of *tomentosa,* etc. Obviously this method is best adapted to species which have large stems. The colchicine treatment has also been found effective and it is more widely applicable than the heteroauxin treatment. As a result of employment of these two agents tetraploid representatives of a wide range of monobasic and dibasic species are available for comparison and for further experimentation.

As regards the monobasic species, the response to tetraploidy in Nicotiana agrees so well with the general features which have come to be recognized as characteristic of the condition that extended comments are unnecessary. The tetraploids exhibit the enlarged growth features and flower size, increased cell and pollen size characteristic of the condition. But while the dibasic amphidiploid species exhibit increased cell and pollen size, thickened tissues, etc., the vigor and total growth attained are inferior to those of the normal species. Furthermore, in the monobasic species, higher polyploidy has an adverse effect similar to that in the amphidiploid species but more pronounced; so that evidently doubling of chromosome number in Nicotiana operates under very definite restrictions.

A survey of autopolyploid effects in Nicotiana seems to give little hope of any direct commercial utilization of the condition. There is a report (Noguti, Oka and Otuka, 1940) that tetraploid representatives of both *Tabacum* and *rustica* have a higher nicotine content than that of the corresponding diploid representatives, which may indicate a special value for some purposes but not for those for which tobacco is normally employed. In fact, it seems to us that the possible value of autopolyploidy has been greatly overestimated. Even in the floricultural field, it is entirely possible that a more sophisticated attitude towards garden products will lead to discrimination against autopolyploids.

Autopolyploids may, however, have very distinct advantages in special breeding programs. A very good

illustration is afforded by the work of F. O. Holmes (1938) on transfer of the mosaic-resistant feature from *glutinosa* to *Tabacum*. *Glutinosa* responds to mosaic infection by production of small, necrotic spots at the locus of infection, whereas *Tabacum* varieties with few exceptions are subject to systemic infection. The direct $F_1$ hybrid between the two species exhibits the non-systemic, necrotic type of infection characteristic of *glutinosa;* but the hybrid is completely sterile, even when backcrossed to the parents. However, the hybrid between these two species which has a diploid set of *Tabacum* and a haploid set of *glutinosa* chromosomes, still exhibits the necrotic type of infection, and it is highly fertile. Such a hybrid may be obtained by crossing tetraploid *Tabacum* with diploid *glutinosa,* but Holmes established it by crossing amphidiploid *glutinosa-Tabacum* with *Tabacum*. From this point the transfer of the character was effected by successive generations of recurrent backcrossing to *Tabacum,* selecting in each generation parents which exhibited the necrotic type of infection. Parenthetically, although the transfer was readily accomplished in this way, interpretation of the results is not entirely clear, for it evidently requires at some point an exchange of segments between a *Tabacum* chromosome and the *glutinosa* chromosome bearing the factor for resistance; but at any rate the results are unquestionable. Availability of polyploids evidently opens up new and more effective means of employing interspecific hybridization in plant-breeding operations.

### AMPHIDIPLOIDY

Not only have heteroauxin and colchicine been employed in obtaining autopolyploids, but they have also been used for producing amphidiploids. Obviously, treatment of decapitated $F_1$ hybrids with heteroauxin will lead to the amphidiploid condition, as a consequence of chromosome doubling in callus shoots, and the same effect may be obtained if $F_1$ seeds are treated with colchicine.

Amphidiploids have also been produced in our investigations by crossing tetraploid representatives of the parental species, but this method has the disadvantage of producing a rather high percentage of unbalanced types with one or more chromosomes fewer or more than the standard number. Usually, however, it is possible to pick out the balanced type without difficulty.

Amphidiploidy, of course, is a polyploid condition of recognized theoretical and practical importance. It has obviously been widely concerned in the evolution of many natural species and in the establishment, mostly up to this time unconsciously, of a number of valuable agricultural and horticultural products. The interest in it arises from the fact that amphidiploidy represents a hybrid condition which is fertile and breeds true for the hybrid features. As a consequence of doubling of chromosome number in a hybrid, each chromosome is represented twice in the amphidiploid, instead of once as in the normal hybrid, and this is the primary condition for normal reproductive behavior.

It has been assumed that amphidiploids are uniformly fertile, especially if the chromosomes of the parental species are so distinct that little conjugation occurs among them in the normal hybrid. We have been astonished, however, to find that a number of those which we have obtained, including those which we hoped would throw some light upon the amphidiploid constitution of *Tabacum,* are completely female-sterile. Specifically, the phenomenon has been observed in four amphidiploids, three of which represent crosses between *sylvestris* and some member of the *tomentosa* group, *viz.,*

4n *sylvestris-Setchellii*
4n *sylvestris-tomentosiformis*
4n *sylvestris-tomentosa*
4n *glutinosa-tomentosa*

Greenleaf (1941) has studied the reproductive phenomena of these amphidiploids. The female-sterility is of a peculiar kind. The meiotic behavior appears to be nor-

mal, at least until the later phases. Chromosomal conjugation is as normal in the macrosporocytes as in the microsporocytes, with 24 pairs in the first division and regular distribution. Failure occurs in embryo sac development, which rarely passes beyond the 2- or 4-nucleate stage, after which degeneration and abortion set in. No fertilization occurs; no seeds are produced. The pollen, however, is good and gives rise to a uniform progeny when used on *N. Tabacum.*

The genetical basis of this phenomenon has not yet been analyzed nor has it been determined how frequently it occurs among Nicotiana amphidiploids. In the case of *tomentosa* the possibility has been investigated that different strains might give different results. Amphidiploids were established from four strains; three by crossing tetraploid *sylvestris* with tetraploid *tomentosa,* and one by the heteroauxin treatment of decapitated $F_1$ plants. Two of them exhibited a very limited fertility, one at about the one per cent. level, the other lower; the other two seemed to be completely female-sterile. It appears that in the two fertile ones occasional embryo sacs develop to maturity. The case is further complicated by the fact that Kostoff (1938), using a different method, has obtained a fertile *sylvestris-tomentosiformis* amphidiploid; but it differs from ours not only in fertility but also in morphological features.

The $F_1$ hybrid of *Tabacum* with 4n *sylvestris-tomentosiformis* or 4n *sylvestris-tomentosa* is fertile; the pollen is about 90 per cent. good, although somewhat less uniform than that of the parental types; but there is a pronounced abortion of ovules. Only about 15 per cent. of the ovules develop into mature seeds, the rest are completely aborted as in the amphidiploid. $F_2$ and backcross progenies have as yet shown no clear-cut segregation ratios for sterility.

These observations certainly demonstrate that fertility is not an invariable consequence of the amphidiploid condition, and they lay some restrictions on the practical and evolutionary significance of the phenomenon.

Another interesting observation relates to the effect of high polyploidy in these amphidiploids. Octoploid representatives of both *sylvestris-tomentosa* and *sylvestris-tomentosiformis* have been established (Greenleaf, 1938), and both exhibit in exaggerated degree the adverse effect of the further increase in chromosome number. They have very thick stems, leathery, misshapen leaves, malformed flowers; and they exhibit cell enlargement out of all proportion to that expected from the chromosome number.

In plant-breeding operations amphidiploids may not only be employed directly, but they may often be used effectively for transfer of factors from one species to another. Thus *sylvestris-Tabacum* and *tomentosa-Tabacum* are so highly sterile as to be difficult subjects from which to secure further progenies, but the hybrid of *Tabacum* with amphidiploid *sylvestris-tomentosa* is fertile enough to be an effective point of departure for transferring factors from *sylvestris* and *tomentosa* to *Tabacum*. And there may well be some factors in these species which are worth transferring. More or less accidentally, we have found that there is a factor for leaf number in the B chromosome of *tomentosa* which when transferred to *Tabacum* adds five or six to the total leaf number.

### Tabacum and Its Amphidiploid Counterparts

As noted above, one of the objects of our investigation was to reconstruct an amphidiploid equivalent to *Tabacum*. For this purpose we have as choices for parental species, on the one hand *sylvestris*, and on the other hand, one of a number of species and forms comprising the *tomentosa* assemblage; *viz.*, *otophora*, *Setchellii*, *tomentosiformis*, and a number of different selections of *tomentosa* itself. At the outset *otophora* was ruled out, because its hybrids with *sylvestris* rarely survive beyond the cotyledon stage. This left the three possibilities which we have already listed, the amphidiploid hybrids of *sylvestris* with *Setchellii*, *tomentosi-*

*formis* and the various forms of *tomentosa.* Of these 4n *sylvestris-tomentosiformis* appears to approach most closely to *Tabacum,* but it still leaves much to be desired. Its female-sterility has already been mentioned. Its morphological features resemble those of *Tabacum,* but it is probably too much to say that they really lie within the range of expression of existing varieties of *Tabacum,* extensive as that range is. Now obviously the discrepancy may arise either from an improper choice of parental species; or as a consequence of alterations which have been incorporated in *Tabacum* since its establishment as a raw amphidiploid. It will probably be difficult to discriminate between these two sources of difference in many instances; but there are some differences which, from their very nature, can only be interpreted as having occurred since establishment of the amphidiploid condition. Obviously if the changes in this category are extensive, it may be impossible to get a raw amphidiploid which would closely approximate existing *Tabacum,* even though it might duplicate the raw amphidiploid from which *Tabacum* has arisen.

Some hint of more fundamental differences than those which are likely to be dependent merely upon a choice of parental types, may be secured from a comparison of *Tabacum* types with corresponding *sylvestris-tomentosiformis* products. With our present experimental techniques, there are numerous ways in which a comparison may be made, but space limits us to a brief exposition of haploid, diploid and tetraploid levels.

Haploid *Tabacum* should be comparable to the normal $F_1$ *sylvestris-tomentosiformis* hybrid; but as a matter of fact it is less vigorous and presents numerous morphological differences. The most significant comparison for our purposes is probably that of meiotic behavior. *Tabacum* exhibits very little chromosome pairing, according to Lammerts (1934) an average of less than one pair per cell. The normal *sylvestris-tomentosiformis* hybrid exhibits a higher average association, about 2.5

bivalents per cell. Evidently, if we may accept this evidence as bearing upon transformation in *Tabacum* since its establishment as a raw amphidiploid, then the process has reduced the effective cross-homology between the two subgenomes of *Tabacum*.

On the diploid plane, the comparison is between normal *Tabacum* and amphidiploid *sylvestris-tomentosiformis*. The morphological differences are much the same as on the haploid level, with the expected increments in size and associated features, characteristic of chromosome doubling. Cytologically the two are closely equivalent, as regards regularity of association and distribution of chromosomes in meiosis. The cross-homologies between subgenomes appear to be only rarely effective in establishing multivalent associations in either one. The outstanding difference is the baffling female-sterility of the amphidiploid.

Some further information may be obtained from crosses between the two. The $F_1$ is highly uniform, which may be taken as confirming evidence of the regularity of chromosomal distribution in the amphidiploid meioses. The morphological features are intermediate, but so close to *Tabacum* that one would probably accept them as representatives of the *Tabacum* assemblage. The cytological behavior of $F_1$ is somewhat lacking in stability. Typically the association appears to be $22_{II} + 1_{IV}$, with the expected variations based upon presence of a translocation complex. Not a great deal of significance can be ascribed to this translocation, because not only are some members of the *tomentosa* assemblage relatively translocated with respect to one another, but *Tabacum* varieties may also exhibit the same relation. There may be some impairment of association, as revealed by a lower chiasma frequency, but it is not great. The evidence on the whole seems to support the conclusion that the amphidiploid is cytologically and genetically equivalent to *Tabacum*, but not of course identical with it.

The existence of numerous genetic differences is shown

by the great variety of recombination products which occur in $F_2$ and other segregating progenies. Taken together with the evidence of absence of any numerous structural differences between the chromosomes of *Tabacum* and the amphidiploid, it may justify the conclusion that alterations in *Tabacum* since its establishment have been largely confined to changes which have not involved structural dislocations.

On the tetraploid level the comparison is between tetraploid *Tabacum* and the doubled amphidiploid, both 96-chromosome types. *Tabacum* gives a normal tetraploid product, somewhat reduced in vigor and in total size; but capable of reaching maturity and of reproducing itself as well as most tetraploids do. The corresponding amphidiploid product, however, is highly abnormal, an excellent example of the adverse effects of too high chromosome numbers. It has thick, misshapen leaves with defective cell-lines, thick stems, stunted growth. Rarely it produces a few misshapen flowers, but no seeds. It seems to have reached the limit of endurance as far as increase in chromosome number is concerned; whereas tetraploid *Tabacum* only shows relatively slight adverse effects at the same level.

Taking all these lines of evidence together they seem to support the conclusion that the alterations which have occurred in *Tabacum* since its establishment as an amphidiploid have tended to transform it in the direction of making it more effectively diploid.

## THE CONSTITUTION OF TABACUM

The relation between the genetic features which characterize present-day *Tabacum* and those of its amphidiploid prototype, can not of course be determined accurately unless we can reproduce the original amphidiploid or something close to it. On the other hand, if the differences are largely due to alterations which have been incorporated subsequently to establishment of the amphidiploid condition our chances of recognizing the raw condition vanish.

*A priori* we can look upon the original amphidiploid as having a highly duplicated genetic constitution, since it arose as a union of two self-sufficient genomes. Are the subgenomes of *Tabacum* any longer self-sufficient? Would they, if separable, be capable of independent existence? The answer, I think, to both questions is no, and the reason for the negative answer is that *Tabacum* is no longer as highly duplicated in its genetic system as a raw amphidiploid must be at its inception. The alterations which have diminished its duplicational completeness must have arisen largely since it became established as an amphidiploid.

Now that we have an amphidiploid which probably is at least closely comparable to the one from which *Tabacum* was derived, it becomes possible to demonstrate some of the alterations experimentally. As an illustration, consider the well-known mammoth growth type of *Tabacum*. In *Tabacum* it is a simple recessive to normal growth; but it is also recessive in crosses to both *sylvestris* and *tomentosiformis*. Under the simplest assumption, therefore, amphidiploid *sylvestris-tomentosiformis* must have duplicate factors for normal growth, whereas present-day *Tabacum* has only a single factor for it. By the use of monosomic *Tabacums*, the analysis has been carried out more precisely. The locus of the $Mm_1$-$mm_1$ factors for normal *vs.* mammoth growth have been shown to lie in the F chromosome, which is a member of the *tomentosa* subgenome. The *sylvestris* subgenome therefore has lost the normal factor, although *sylvestris* still has it.

More precise information is afforded by proper crosses of *Tabacum* with the amphidiploid. The procedure is rather complicated, but the results are so clear-cut as to appear worthy of presentation. An $F_1$ haplo-F hybrid is obtained by crossing haplo-F *Tabacum* with the amphidiploid. It has a single chromosome-F derived from *tomentosiformis;* the rest of the chromosomes are paired off, one member *Tabacum*, the other member a corre-

sponding *sylvestris* or *tomentosa* chromosome. For the chromosomes of interest in this analysis the situation may be formulated as follows (the primed symbols representing *sylvestris* and *tomentosiformis* chromosomes):

| normal $F_1$ | | haplo-F $F_1$ | |
|---|---|---|---|
| F-$Mm_1$ | F′-$Mm_1$ | $F_0$ | F′-$Mm_1$ |
| X-$mm_2$ | X′-$Mm_2$ | X-$mm_2$ | X′-$Mm_2$ |

When the haplo-F $F_1$ type is now crossed with mammoth *Tabacum*, the following results are secured:

Parents

| haplo-F $F_1$ ♀ | | | mammoth *Tabacum* ♂ | |
|---|---|---|---|---|
| $F_0$ | F′-$Mm_1$ | × | F-$mm_1$ | F-$mm_1$ |
| X-$mm_2$ | X′-$Mm_2$ | | X-$mm_2$ | X-$mm_2$ |

Progeny

| F-$mm_1$ | F′-$Mm_1$ | F-$mm_1$ | F′-$Mm_1$ | $F_0$ | F-$mm_1$ | $F_0$ | F-$mm_1$ |
|---|---|---|---|---|---|---|---|
| X-$mm_2$ | X′-$Mm_2$ | X-$mm_2$ | X-$mm_2$ | X-$mm_2$ | X-$mm_2$ | X-$mm_2$ | X′-$Mm_2$ |
| diplo-F normal | | diplo-F normal | | haplo-F mammoth | | haplo-F normal | |

Note that the diplo-F plants in this progeny are all normal; and that the haplo-F plants are of two kinds, half normal and half mammoth, whereas a *Tabacum* cross of this kind always gives simply diplo-F normal and haplo-F mammoth plants. The evidence warrants the conclusion that an $Mm_2$ factor is carried into this hybrid by an as yet unidentified chromosome, represented by X in this analysis. By the use of monosomic analysis it should be possible to identify specifically the *sylvestris* chromosome which bears this normal factor and thus to establish a genetic cross-homology between *sylvestris* and *tomentosiformis*. Meanwhile note that this duplicate normal factor may now be transferred from the amphidiploid to *Tabacum* with perfect assurance of avoiding any confusion with the normal factor of *Tabacum* present in the F chromosome by backcrossing these haplo-F normal plants to mammoth *Tabacum*.

The objection may of course be raised that we can not be certain that all varieties of *Tabacum* have lost this

second normal factor, but as we multiply similar instances this objection seems to lose its cogency. As another example there is an asynaptic condition in *Tabacum,* a simple recessive to normal meiotic behavior. Obviously both of the parental species of the raw amphidiploid must have had a complete set of factors for normal meiotic behavior, consequently, the normal allelomorph of asynaptic must have been duplicated in the raw amphidiploid. Since the asynaptic factor in *Tabacum* is located in the A chromosome, a *tomentosa* homologue, the duplication again must have been eliminated from some chromosome of the *sylvestris* subgenome.

Similar arguments apply for a simple recessive white-seedling character. Here the locus is found to lie in the T chromosome, a *sylvestris* homologue, and the duplication must consequently have been eliminated from some member of the *tomentosa* subgenome. From these and other examples, the conclusion appears to be ineluctable that one of the features of transformation of the raw amphidiploid into the finished product has been the elimination of some of its duplications.

Some information as to this problem may also be obtained from a comparison of the sensitivity of monobasic and amphidiploid species to deficiency. In plants, monobasic species in the main are so sensitive to loss of segments that even the smallest deficiencies usually lead to lethal gametophytic effects, so that the conditions are not even transmissible. On the contrary in dibasic amphidiploid *Tabacum,* $(n-1)$ gametophytes are viable for subtraction individually of at least 21 of the 24 chromosomes of the *Tabacum* complex and probably for all of them, although in highly varying proportions, and even degrees of deficiency as high as $(n-4)$ may sometimes function. This difference between monobasic and amphidiploid species seems unquestionably a consequence of duplication, and in this instance must indicate that many of the duplications still exist in the transformed amphidiploid species.

However, we have found, at least for some of the chromosomes, that while 23-chromosome gametophytes may be viable, the corresponding $23_{II}$ sporophytes may not be. This is probably true for all chromosomes of the *Tabacum* complex, but satisfactory experimental evidence is available for only a few. In higher polyploids, such as the tribasic oats and wheat, it is not necessarily true, but in these instances the sporophytes often exhibit curious and illuminating defects, as for example the asynaptic feature of a $20_{II}$ type in oats (Huskins and Hearne, 1933).

The genetic basis of the inviability of $23_{II}$ sporophytes has been investigated rather fully for the F chromosome. It is found that deficiency for one arm, let us say the left, recognized by the appearance of a coral flower color and other associated qualitative features, does not lead to inviability. Deficiency for the right arm, however, is zygotically lethal. The most rigorous proof comes from a set-up in which both arms of the F chromosome have been translocated. The zygote is then viable only if a small additional ring-shaped fragment derived from the F chromosome is present. During development of such a sporophyte the ring fragment is lost sporadically. The result is production of necrotic areas most plainly seen in leaves and flowers. Evidently the vital portion of the chromosome is here restricted to a very small part of the chromosome, probably a single factor, located near the centromere, since the ring chromosome which contains it is very small. Similar results have been obtained with the C chromosome; and it is perhaps reasonable to assume that these results are a pattern of those which may be expected from any of the chromosomes.

Now it is possible to set up a viable genetic system which completely lacks an F chromosome. Unfortunately the female sterility of amphidiploid *sylvestris-tomentosiformis* makes it impossible simply to set up a nullo-F $23_{II}$ condition of it and to observe its results. But it is possible by crossing haplo-F *Tabacum* with *sylvestris* to

secure a nullo-F 35-chromosome hybrid, which exhibits $12_{II} + 11_{I}$ instead of $12_{II} + 12_{I}$ as in the normal hybrid. It has coral instead of carmine flowers and differs in a number of ways from the normal hybrid, but it is a viable vigorous type. Our analysis of this situation follows the same pattern as before. Since in *Tabacum* we have shown that nullo-F is inviable as a sporophyte, probably because of the elimination of a single vital factor, the viability of this nullo-F hybrid must point to introduction of that factor into the system by *sylvestris,* and following up the argument, it must indicate that an originally duplicate condition has been reduced to a simple condition by loss of this factor from the *sylvestris* subgenome. Now since we find similar conditions obtaining for many, if not all of the chromosomes of the *Tabacum* complex, in the aggregate this simplification of the genetic constitution must have involved very extensive alterations.

No attempt has been made up to this time to describe the nature of the transformation process beyond the fact that it eliminates some of the duplications present in the original amphidiploid. It has been shown that an amphidiploid may endure extensive deficiencies, but there is little evidence to suggest that the process we have described depends upon actual elimination of genetic material. In fact the regular meiotic behavior of $F_1$ crosses between the raw amphidiploid and the transformed product probably indicates that chromosomal dislocations have played a rather minor part in the process. It would appear therefore that simple gene mutation probably was the most likely agency, although in that event we are left floundering for an explanation of the reduction in cross-homology of haploid *Tabacum* as compared with $F_1$ *sylvestris-tomentosiformis.*

Space forbids extensive exploration of the theoretical consequences of some of the ideas set forth above. We must content ourselves with a statement of three rather obvious conclusions.

First, the possibility of duplicating an established

amphidiploid species precisely appears to be rather slight. It will be more profitable to direct attention toward the nature and extent of the transformations which have occurred subsequent to establishment of the amphidiploid condition.

Second, if the raw amphidiploid contains extensive series of duplications which have been reduced to simpler conditions in the transformed product, it should be possible by employment of the monosomic technique to determine cross-homologies between the chromosomes of the contributing genomes.

Third, since the process of simplification of genetic constitution in amphidiploid species appears to have affected extensively duplicated vital factors, a severe restriction is imposed upon the freedom of recombination between the monobasic parental and the derived amphidiploid species. No restriction is imposed upon transfer from the monobasic to the derived amphidiploid species, presumably any segment in the amphidiploid may be substituted by the corresponding segment from the appropriate monobasic species. But transfers from the amphidiploid to the monobasic species will frequently give non-viable or defective products, since the segments may contain some of the transformation products to which attention has been called.

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## DISCUSSION

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OF AGRICULTURE

THE following are three lines of study on polyploidy in the genus Nicotiana in which we are engaged in the Division of Tobacco Investigations.

The first involves the occurrence of polyploidy in hybrids between *N. Debneyi* Domin. and *N. tabacum* L. without using artificial means of induction. This cross is easy to make and a number of viable seed are formed which germinate well but, when the seedlings have grown to be about a quarter of an inch tall, over 99 per cent. of them dies. Of the few plants that live and grow to maturity, some are partly fertile. This material was turned over to Mr. Sanford Newman to study cytologically. He found that the surviving $F_1$'s frequently formed dyads, in some plants as high as 70 per cent.

The $F_2$ generation was quite uniform but showed some variability, obviously due to occasional chromosomal loss in micronuclei formed along with the dyads. This $F_2$ was similar to the progeny of an amphidiploid *Debneyi* × *tabacum,* produced by colchicine treatment. These results supported the cytological evidence that the mechanism of dyad formation is dependent on asynapsis and is replaced by the regular formation of tetrads when chromosome pairing is restored. Dyads have been found to occur occasionally in Nicotiana species crosses; but it is unusual to have them formed regularly and frequently so as to produce relatively high pollen fertility, seed set and plentiful amphidiploid progeny of the $F_1$.

Investigations on the *Debneyi* × *tabacum* cross were begun originally and are being continued by the pathologists who are working to transfer the factors for resistance to blue mold from *Debneyi* to the cultivated *tabacum.*

Secondly, we are using polyploidy as a means of producing a strain which will yield large amounts of anabasine, an alkaloid with insecticidal properties. It has been found to be present in small amounts as the main alkaloidal constituent in only two species of Nicotiana, namely: *N. glauca* R. Graham and *N. Debneyi.* From crosses between *glauca* and the cultivated species which have a high concentration of nicotine, $F_1$'s and amphidiploids were produced which contained mostly anabasine with small amounts of nicotine. A fairly fertile, pure breeding amphidiploid strain of *tabacum* × *glauca* was developed which has a higher concentration of anabasine than *glauca* and also has an improved growth habit. It may be possible to produce still better anabasine-yielding strains by combining the best qualities of *N. glauca, N. tabacum, N. rustica* L. and possibly *N. Debneyi* into new fertile types with the aid of colchicine as a means of doubling chromosomes in certain hybrids among these species.

Results have shown that chromosome doubling in the different species themselves produces markedly different effects. As Dr. Clausen has pointed out, autopolyploids of dibasic species with 24 pairs of chromosomes, such as *N. rustica* and *N. tabacum,* are less vigorous than their undoubled progenitors. This does not hold for all species with 24 pairs of chromosomes, however, since 4n *Debneyi* is as large as the diploid ($2n = 48$). Tetraploids of the monobasic *N. glauca* are less vigorous than plants of the normal species. Since *glauca* has unusually large chromosomes for the genus Nicotiana, it appears that certain species with large chromosomes as well as certain ones with a high number of chromosomes may be adversely affected in somatic development by chromosome doubling.

In the third place, we are using induced polyploids as a basis for obtaining extra chromosomal types of *N. Langsdorffii* Weinm. and *N. Sanderae* hort., two species which have 9 pairs of chromosomes. It has been deter-

mined, by means of statistical analysis and linkage with color genes, that the difference in corolla size between *Langsdorffii* (small flower) and *Sanderae* (large flower) is controlled by many genes, apparently well distributed in the chromosomes. Plants with single extra chromosomes from *Langsdorffii* on the background of the $F_1$ with *Sanderae* have been obtained and all, so far, have had smaller corollas than the $F_1$, due to genes for small size that were added. By using analyses of this sort with favorable material, information can be gained on genes affecting quantitative characters which are located in individual chromosomes.

# INDUCED POLYPLOIDY IN FLORICULTURE[1]

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## INTRODUCTION

THE comparatively recent discovery of the ease with which the chromosome number in plants may be doubled by using colchicine appears likely to greatly stimulate interest in flower breeding. As a rule tetraploids have larger flowers and fruits than diploids, and in addition, as pointed out by Müntzing (1936), they usually bloom later and thus may extend the flowering season in some localities. All these characteristics have important bearing on the commercial value of many ornamental plants.

The work of Nebel and Ruttle (1938) in inducing tetraploidy by using colchicine in marigolds, petunias, snapdragons and pinks, indicated some of the possibilities in ornamentals. Blakeslee and Avery (1937) reported induced tetraploidy in Portulaca, and Levan (1939) both tetraploidy and octoploidy in Petunia. Later Emsweller and Brierley (1940) secured tetraploid forms of *Lilium formosanum* by using colchicine, and recently Weddle (1940) reported induced polyploidy in Chrysanthemum and Calendula.

In this paper consideration will be given to the more recent investigations carried on by the senior author and his colleagues at the United States Horticultural Station, Beltsville, Md., and by the junior author at the Agricultural Experiment Station, Geneva, N. Y.

[1] Approved by the Bureau of Plant Industry, U. S. Department of Agriculture, and the Director of the New York State Agricultural Experiment Station, as journal paper 437, March 19, 1941.

### Experiments at Beltsville

The greater portion of the work at Beltsville has been with those ornamental plants that are usually propagated asexually. The only normally seed-propagated material we have treated is the double-flowered stock, *Matthiola incana.* Asexually propagated ornamental plants have a decided advantage since the difficulties of sterility and heterozygosity are not factors in propagation. Among the asexually propagated types included in our experiments were a variety of perennial phlox, several varieties of chrysanthemum, one of the African violets, several begonias and 11 species of *Lilium.*

All lilies thus far examined have $2n = 24$ chromosomes except the common *Lilium tigrinum,* which is a triploid with 36. Attempts to induce tetraploidy in a number of *Lilium* species by treating seed and seedlings failed. A similar experience with gladiolus species has been reported to us verbally by Dr. Ronald Bamford, of the University of Maryland. The first lily tetraploids were secured at Beltsville by immersing the growing point of *L. formosanum* in a colchichine solution which checked terminal growth for several months. Eventually a number of bulblets were produced in the axils of leaves growing from the treated region. Cytological examination of root tips from most of these bulblets showed tetraploidy to be present. In some instances, however, variation in stomate size on the same leaf indicated that some of these plants were probably mixoploid.

The tetraploid formosanum plants were 2 to 4 weeks later in blooming and produced flowers that were about 25 per cent. larger than those produced from diploid bulbs from the same soma. The tetraploid plants were shorter than the diploids, their leaves were broader, thicker and darker green. The size of stomates was variable from plant to plant and even on the same plant, but as a whole those on the tetraploids were larger than those on diploids. Fertility of the tetraploids varied from 0 to 15 per cent. of that normally occurring in

diploids. Of special interest was the variation in fertility of tetraploids produced by doubling the same soma. In one group of 9 sister tetraploids only 1 set seed.

The same method of treatment, later applied to several other lily species, including *Lilium longiflorum, L. candidum* and *L. testaceum,* failed because none produced top bulblets. In a further experiment with *L. longiflorum* the fleshy bulb scales which are commonly used to propagate this species were immersed in colchicine. A rather extensive series of treatments was applied to a large number of scales from a clon developed from a single bulb several years ago. In many instances 3 or 4 bulblets developed at the base of one scale, one or more of which were polyploid and the others diploid. A large number of aberrant plants resulted from these treatments. Cytological studies now under way indicate that while tetraploids have been produced it sometimes happens that portions of a plant remain diploid or may even be octoploid. Such plants composed of tissues containing different chromosome numbers are commonly called mixoploids. A considerable variation is evident among the affected plants produced from treated scales, whereas plants from untreated ones are exceedingly uniform. The variations involve chiefly leaf shape, growth rates, areas lacking chlorophyl and vigor. No flowers have been produced as yet on the longiflorum tetraploids, but their buds are definitely larger than those on diploid plants. The methods and results of the experiment with the bulb scales will be reported later when the cytological investigations have been completed.

As soon as polyploidy had been successfully induced in *L. longiflorum* by the scale-treatment method similar treatment was applied to other species, and from stomate measurements it appears that polyploidy has been induced. The species treated were: (1) *Lilium auratum,* (2) *L. speciosum,* (3) *L. candidum,* (4) *L. testaceum,* (5) *L. tigrinum,* (6) *L. pardalinum,* (7) *L. regale,* (8) *L. davidi* and (9) *L. hansoni.* Since most of these species

grow very slowly, it is not likely that any flowers will be secured for another year or two.

The perennial phlox variety Miss Lingard has been shown to be resistant to septoria leaf spot (Post, 1938). A few observations of pollen mother cells of this variety disclosed some chromosome fragments and other meiotic irregularities which may explain the 36 per cent. abortive pollen reported by Post as well as the very high sterility. Colchicine treatments in 1940 of this variety have produced typical polyploid effect with enlarged stomates, but none of the plants has bloomed.

In 1939 several lots of chrysanthemum seedlings were given colchicine treatments. The young, vigorously growing seedlings were treated by immersion of the growing point. Following treatment a number of plants produced side branches with large thick leaves, heavy stems and large stomates. These branches were rather compact and produced strikingly abnormal flowers. On all affected branches there was a decrease of flower size, and in most cases a complete abortion of anthers. Sometimes the individual florets proliferated and formed various floral monstrosities. All the flowers were completely sterile. The results secured by Weddle in doubling the chromosome number of chrysanthemum seedlings differed somewhat from ours. His doubled plants produced some seed and the flowers were normal, but he concluded that little net improvement had resulted from higher polyploidy in the chrysanthemum.

The so-called African violet, *Saintpaulia ionantha,* has been colchicine treated at Beltsville and some leaf cuttings appear to be affected. All treated cuttings exhibited a severe check in growth and some of the new plants developing from treated tissue show indications of polyploidy. Leaf cuttings of several treated begonias are also producing plants that appear promising. It is probable, however, that many of these leaf cuttings may prove to be chimeral types.

The chromosome number of three races of stocks,

*Matthiola incana,* has been doubled at Beltsville. This species produces two types of plants, one bearing single and the other double flowers. The double-flowered plants set no seed because they produce no stamens or pistils. Singleness is a simple monogenic dominant over doubleness, and there are three recognized genotypes in the single-flowered plants. These are homozygous single, heterozygous single and so-called eversporting single. The eversporting single produces about 53–55 per cent. double-flowered plants because of a gametic lethal carried in the same chromosome as the singleness (S) gene (Winge, 1931). The lethal gene inactivates the pollen carrying it, so that only those transmitting the recessive double (D) gene function. In the eggs a few of those carrying the lethal and (S) genes are supposedly inactivated, thus decreasing the expected number of single (S) eggs and causing the unbalancing of the 1 : 1 ratio.

Young seedlings from lines inbred 5 generations, and representing each of the 3 genotypes, were colchicine treated in 1940. Within each genotype, plants with two or more polyploid branches were produced. These branches were sometimes pure polyploid but a large number were mixoploid types. In many instances both diploid and polyploid branches were produced on the same plant. Of considerable assistance in making preliminary identification of polyploids was the modification of glandular hairs on leaves and young buds. On affected branches these hairs were larger, spaced farther apart and considerably more branched. When two polyploid branches were present, one was self-pollinated and the other crossed back to the diploid. None of the triploid crosses produced seed, although all induced fruit setting. Some of the polyploid branches proved to be moderately self-fertile and there were instances where, on the same plant, one set seed whereas another from the node above or below failed to even form fruits. Only one branch was examined cytologically and the tetraploid number of chromosomes (28) was determined. The pollen of all

branches assumed to be polyploid because of the modification of glandular hairs on leaves and young buds was examined. Some of these branches proved to be tetraploid; but others had small uniform pollen grains and complete fertility in no way different from known diploids, and probably were periclinal chimeras.

Some of the double-flowered plants, when treated, also produced polyploid branches. The flowers on such branches were slightly larger than those on the diploid branches of the same plant. Since all affected branches exhibited the well-known colchicine "shock" or retardation effect, it was felt that their flowers were probably not exhibiting their full development.

Of special interest was the progeny of a tetraploid eversporting single-flowered plant, since it carried a double dosage of the lethal gene. The data so far accumulated indicate that the double : single ratio has not been affected. Populations from all the fertile polyploids are now being studied and will be reported on in a later paper.

### Experiments at Geneva

The more recent polyploidy work on ornamental plants at the New York Agricultural Experiment Station has dealt chiefly with snapdragons and marigolds, both of which are normally reproduced by seeds, but which may be increased by cuttings. This is especially true of snapdragons, which were generally propagated by cuttings some years ago before rust became such a problem. Rooting marigold cuttings is more difficult and, although they have been increased in this way, such practice does not appear promising.

Colchicine treatments of snapdragons at Geneva have produced tetraploids of the following varieties: Orange Shades, Red Shades, two types of Harmony Shades, Autumn Glow, Torchlight, Pinkie, Rose Pink Cheviot, White Wonder, Alaska, Early Sunlite, Golden Rod, Velvet Beauty, and three unnamed varieties, two of them resistant to form 1 of snapdragon rust. The majority

of these are now growing in the second and third generations.

The tetraploid snapdragons have larger flowers than the diploid parent with corollas more ruffled and of a deeper shade. Flowers of most varieties are characterized by small papillae on the corolla surface near the margin. This is not true, however, of Early Sunlite and two unnamed varieties. The plants are of approximately the same height as the diploid but the stems are more sturdy, the leaves thicker and of a darker green color. In most instances the flower spikes tend to be shorter than those of the diploid, this being especially true for Velvet Beauty. The flowers of some varieties open poorly, others open as well as the diploids.

In most of the tetraploid snapdragons produced and grown at Geneva the fertility is greatly reduced, only a few seed being obtained. In some instances, such as tetraploids of Orange Shades and White Wonder, large seed capsules developed but the seeds remained small and did not germinate. In most varieties not even the seed capsules develop. Occasionally a number of seed set on a plant which is usually highly sterile. All tetraploid varieties set seed on being crossed with other tetraploid varieties.

Additional information on fertility of tetraploid snapdragons was secured last summer when the senior author visited a planting in California of approximately 3 acres of tetraploid plants representing various species of flowers. These plants were in a section where the climate is favorable for flower-seed production. There were about 10 races of tetraploid snapdragons. Permission was obtained to examine pollen of these races; only two showed a fair amount of good pollen. At the end of the season the grower kindly furnished us with a report from which we quote, "Not only was there very little snapdragon seed produced on open pollinated plants, but even with careful, diligent hand pollination, only two tetraploid strains set seed in any appreciable amount."

At Geneva, hybrids between tetraploids of the following varieties have been successfully made and grown to maturity. Velvet Beauty was crossed with the following varieties: Red Shades, Early Sunlite, Rose Pink Cheviot, Orange Shades, Pinkie and Torchlight. Pinkie has been crossed with Rose Pink Cheviot, Torchlight and Early Sunlite; Orange Shades, with Alaska and Early Sunlite, and White Wonder, with Rose Pink Cheviot.

The hybrids between the tetraploids grown at Geneva were vigorous plants, with flower size equal to or greater than that of the tetraploid parents, and with longer spikes. Of special interest, however, was the fact that in the first generation all were highly fertile, regularly setting large capsules of good seed. Tetraploid Rose Pink Cheviot × tetraploid Pinkie continued fertile in the second generation, the plants being uniform with no observed segregation. It is not yet known whether the fertility and vigor will remain unimpaired through succeeding generations.

All crosses with tetraploid Velvet Beauty produced plants bearing red flowers. The shade of red and other plant characters varied with the alternate parent. Certain crosses with tetraploid White Wonder had flowers which did not open as well as crosses with tetraploid Early Sunlite. Early Sunlite does not bear papillae on either the inner or outer surface of the upper lip of the corolla as do other tetraploids. The crosses Velvet Beauty × Early Sunlite and Pinkie × Early Sunlite bear small papillae on the corolla. The cross Orange Shades × Early Sunlite bears many large papillae on the inner and outer surfaces of the upper lip as did the Orange Shades parent.

Both diploid and tetraploid Red Shades are highly resistant to form 1 of the snapdragon rust whereas the same forms of Velvet Beauty are highly susceptible. Both diploid and tetraploid hybrids between Red Shades and Velvet Beauty are highly resistant.

A tendency to doubleness is particularly noticeable in

many snapdragon varieties. This seems more pronounced in the tetraploid than in the diploid. For example certain of the tetraploids from White Wonder were almost entirely double as compared with the small amount of doubleness in the diploids. Similarly the tetraploid of White Wonder × Early Sunlite shows considerable doubleness.

Diploid hybrids between many of the snapdragon varieties tend to be more vigorous than either parent. This also applied to the tetraploid hybrids and in particular to the tetraploid hybrid from the crossing of tetraploid Velvet Beauty and Red Shades and of tetraploid Pinkie and Rose Pink Cheviot.

Tetraploid hybrids produced through doubling first generation diploid hybrids corresponded to hybrids produced by crossing artificial tetraploids from the same parental stock.

Triploids were also produced by crossing tetraploids with diploids of the same variety and with different varieties. Their flowers showed some tetraploid characters, but were smaller than those of the tetraploid parent. They were partially fertile and may be promising in the production of diploid variants from which new tetraploid types may be derived.

Treatment with colchicine appears to have induced a higher than normal percentage of diploid somatic mutations in both snapdragons and marigolds. The types described here occurred in both and in certain cases probably represented the loss or gain of a chromosome, but in some the chromosome number was unaltered. A treated diploid snapdragon plant of the variety Orange Shades which bore orange-pink flowers gave rise to a mutant branch bearing yellow flowers with a soft pinkish cast on the outside of the corolla. This mutation bred true in the first generation, and the chromosome number was diploid (16). Another interesting mutant branch appeared on a treated plant of unnamed material. The branch bore peloric rather than the normal zygomorphic

flowers, and this character was present in later generations. The chromosome number was unchanged.

From one treatment a plant was obtained which grew with exceptional vigor and bore flowers larger than any previously obtained. The odor was also noticeably much stronger. Cytological examination showed the plant to be diploid. The chromosome number was then doubled by colchicine treatment of cuttings and the flowers produced were much larger than any yet seen on any other tetraploid. The pollen also tended to be larger than normal.

It must always be remembered that these changes may represent fortuitous mutations, and this possibility is being borne in mind. The senior author has had considerable experience with snapdragons and has grown and observed many thousands of plants. Changes from zygomorphic to peloric as well as color mutations have been observed several times. Weddle also reported on apparently high frequency of color mutants on his colchicine-treated chrysanthemums.

Mutations also occur in both snapdragons and marigolds which can definitely be attributed to the loss or gain of one or more chromosomes. Thus a treated diploid snapdragon gave rise to branches bearing flowers with the upper lip of the corolla much wider than normal and having a third lobe. The chromosome number was 17. Another aberrant seedling from a treated Afterglow plant bore completely sterile bright crimson flowers having short wide corolla tubes. The chromosome number was 22. A variant with 36 chromosomes was produced from treating a seedling from the cross Velvet Beauty diploid × Velvet Beauty tetraploid.

The more recent tetraploid marigolds made at Geneva show definitely that form and quality depend upon the variety doubled. Thus the old Guinea Gold tetraploids were much smaller than new ones of other superior varieties.

The triploid hybrids between diploid African marigold

with 24 chromosomes and tetraploid French Dwarf with 48 chromosomes are highly sterile. Colchicine treatment of this hybrid produced a hexaploid with 72 chromosomes which was fertile.

The cross between the induced African tetraploid and the tetraploid French Dwarf was highly sterile, but had very good flower size, dwarf growth habit, and the orange color was dominant over red in the first generation. The hybrids differed widely depending on the male and female parentage. A large yellow tetraploid which was highly male sterile gave rise to an especially vigorous tetraploid hybrid when crossed with Robert Beist. Flaming Fire by other tetraploids also produced very favorable hybrids.

Among the seedlings from one of the highly sterile hybrids between the African tetraploid and the French Dwarf, each with 48 chromosomes, there appeared a small, weak dwarf plant which had 24 chromosomes rather than the expected 48 or near 48. The 24 chromosomes were probably derived some from the French Dwarf and others from the African tetraploid. It is an unusual haploid type and has been called an allopolyhaplont. The plant was not a typical dwarf or dwarf hybrid but was smaller, less vigorous, and highly sterile. Seven plants from open pollinated seed were obtained, only five of which developed to maturity. Two of the five were tall (40 inches) but more slender than plants of Guinea Gold, and the leaves and flowers were characteristic of dwarf types. The chromosome number in one was 24, in the other 25. Two of the remaining three plants were about 24 inches tall and had small flowers; the fifth plant produced large, heavy, single flowers with thick ligules.

Both of the two taller plants were self-fertile and gave rise to a number of albino seedlings which failed to develop, and to normal seedlings which produced plants varying from extreme dwarfs to typical Guinea Golds. Time of maturity, habit of growth, height and leaf and flower size, shape and odor, all varied from plant to plant.

The color ranged from orange to orange-yellow to lemon-yellow. Certain of the plants in particular had a very favorable habit of growth in that they were of medium height and possessed strong, low-branching stems which made for a well-rounded full plant capable of bearing a profusion of blooms. Treatment of part of the populations with colchicine produced tetraploids with a wide range of variation in the above-mentioned characters.

The progeny of most of the tetraploid marigolds produced by treatment of Guinea Gold was uniform in the first generation unless cross-pollination occurred. One of the Guinea Gold marigolds in 1937 and apparently tetraploid, as indicated by increased pollen size, produced an exceedingly variable progeny which appeared to be tetraploid. One of these plants gave rise to a progeny of only three plants, one of which was tetraploid and produced a normal tetraploid population. The other two were dwarf, one bearing single and the other double flowers. The latter two plants each had 36 chromosomes, indicating possible hybridization with a diploid, although the plants did not appear to be typical triploids. The progeny of the single-flowered dwarf was extremely variable. One of its seedlings was short and stubby with small flowers and it had 24 chromosomes. Selfed seed from this plant produced a variable population which contained 25 per cent. fasciated plants.

## Discussion

It is still too early to evaluate properly the ultimate benefits to flower breeding which may be expected from colchicine or other induced polyploidy. In many ornamental plants there is a definite need for new types with larger flowers, later blooming habit, and other characteristics usually associated with tetraploidy. Many of the finest varieties in some groups are known to be natural tetraploids, some of which are fully fertile, whereas others still exhibit some degree of the sterility commonly associated with auto-tetraploidy. Among the daffodils,

three widely grown, large-flowered varieties, King Alfred, Olympia and Van Waveren's Giant, are tetraploids, and exhibit the usual sterility common in auto-ploids. King Alfred and Van Waveren's Giant do set some seed, but Olympia is reported to be completely sterile. Nagao (1933) reported variable quadrivalent formation in both King Alfred and Olympia and believes they are both auto-tetraploids. Similar instances could be cited in other groups of flowering plants.

In the colchicine-induced auto-tetraploids described above there has been a very definite increase in flower size in marigolds, snapdragons, lilies, petunias, portulacas, calendula, stocks and phlox. This was also true of clarkia and annual delphinium that the senior author observed in California in 1940. Just recently Dr. Ronald Bamford at the University of Maryland has secured what appears to be a tetraploid *Gladiolus tristis,* from colchicine treatment of cormels. This is a diploid species of Gladiolus and while chromosome counts have not yet been made, pollen size and other characteristics strongly indicate that the chromosome number of the diploid Gladiolus has been doubled. The flowers tend to be larger than on untreated checks, but the number so far produced is too small to show any significant difference. In chrysanthemums, the flowers produced at Beltsville on colchicine-treated plants were sterile, very abnormal, and smaller than on untreated checks. Those produced by Weddle at Ithaca were large, normal and set some seed. Chromosome counts on the chrysanthemums were not secured on the Beltsville material and it is not known if we both were working with material having the same number. Chrysanthemums are very variable in all characters including fertility, and the $2n$ chromosome number of various species is known to range from 18 to 90, with most of the intermediate chromosome multiples already reported. Variable results from colchicine treatments are to be expected from such a heterotypic genus.

The sterility problem in seed-propagated auto-tetra-

ploid flowers must also be carefully considered in all practical attempts at utilization of any new material. As pointed out earlier in this paper, sterility in the induced autoploids ranges from approximately 50 to 100 per cent. There has been considerable variation in seed setting on tetraploids of different varieties of the same species. It appears possible then that more highly fertile tetraploid races may be obtained merely by selection. Randolph (1935) reported similar differences in fertility in lines of auto-tetraploid maize, and now has developed by selection strains that are fairly fertile.

The high fertility secured by the junior author from crossing two unrelated self-sterile tetraploid varieties suggests another approach to the sterility problem except for certain irregularities attributed to imperfect parental meiosis. The first generation progeny of these fertile hybrids has been very uniform, which strongly suggests the behavior of an amphidiploid. As yet meiosis has not been studied in this material; therefore nothing can be said concerning chromosome pairing. The high sterility and bad pollen of the original tetraploids are indicative of the probable formation of quadrivalents. Crosses are now being made between unrelated tetraploid stocks (*Matthiola incana*) to determine whether greater fertility may be secured. Cytological examination of some of the tetraploid parents has shown that quadrivalents are formed in varying numbers.

In some varieties of flowering plants that are readily propagated asexually, complete sterility may be highly desirable. In some instances, such as snapdragons and delphinium, the flower spikes deteriorate rapidly after the lower flowers become pollinated. This is an important consideration to a florist, and many good growers of snapdragons make every attempt to keep large insects, especially bumble-bees, out of their snapdragon house to prevent pollination. Recently the senior author observed an unusually large-flowered snapdragon growing in a greenhouse at Denver, Colo. The grower pointed out that

it set no seed and was an excellent keeper as well as large flowered. Root tip examinations made by Dr. Powers at the Cheyenne Horticultural Station of the Bureau of Plant Industry proved it to be tetraploid. The grower has been propagating it asexually for some years, and its exact origin could not be determined.

Among ornamental plants there are many highly desirable forms that are known to be triploids. An outstanding example is the common tiger lily (*Lilium tigrinum*). Here there is another potential use for tetraploid ornamental plants in producing triploids which may have some valuable characteristics. In this same connection it is also interesting to note that some of the cultivated varieties of iris are aneuploids as reported by Randolph (1934).

Diploids are known to develop regularly from tetraploid maize. These behave as normal diploids. The diploid marigold derived from the tetraploid hybrid between the African induced tetraploid and the natural tetraploid French Dwarf possesses chromosomes of both parents. Its progeny therefore carry characters in varying degrees of both parental species. From this plant therefore it should be possible to select favorable intermediate types which can be fixed either by selection or by repeating the process of chromosome doubling, thus producing other tetraploids with a wider range of characters than those derived from pure Guinea Gold stock.

It appears that polyploidy in its many forms has already played an important rôle in the development of many cultivated ornamental plants. In general it is not possible to predict just what may be the value of a new tetraploid, triploid, or aneuploid. Many of our cultivated ornamental plants are already polyploids and may already have reached the maximum in quality as a result of chromosome duplication. This may be true for commercial varieties of gladioli, all of which so far examined (Bamford, 1935) are already tetraploid. Likewise the commercial varieties of dahlias, roses, and chrysanthe-

mums are all highly polyploid and are likely to prove disappointing if higher degrees of polyploidy are induced.

From the purely practical point of view commercial flower-seed growers cannot be expected to be interested in highly sterile, seed-propagated ornamentals no matter how fine the quality. The low fertility, however, may be solved as more data are accumulated on the ever-increasing number of induced tetraploids.

A brief summary of the already known advantages of induced polyploidy in flowers is added:

1. The induced tetraploids may be desirable in themselves for size of flower, quality of stem or time of blooming—marigolds—*Lilium formosanum.*

2. The tetraploid varieties if self-sterile may be crossed with other tetraploid varieties to secure fertile amphidiploids—snapdragons.

3. Fertile amphidiploids may be produced from sterile interspecific hybrids—marigold—mint—which may be of value in themselves or may be used for backcrossing to their respective parents.

4. The tetraploids may be used for making interspecific hybrids in polyploid genera tobacco and marigolds.

5. The amphidiploid varieties (snapdragon) have again been crossed to produce plants with four different sets of parental chromosomes. These will give different genetic ratios from diploid hybrids.

6. A study of inheritance within both varietal tetraploids and their hybrids (amphidiploids) is showing to what extent certain tetraploid characteristics are the result of a new gene balance and to what extent the new gene complex will segregate in successive generations or whether the characters of the tetraploid gene complexes are inherited as physiological units.

7. Triploids produced by backcrosses of tetraploids to diploid parents and other diploids and the reverse are partially fertile (snapdragons) and may produce aneuploid lines of assistance in analysis of parental diploid and tetraploid stocks.

8. Aneuploids of value in themselves and in an analysis of the group are obtained directly from treated lines.

9. The diploids obtained from amphidiploids (marigold) and carrying characters of both parents is a means of building up an intermediate series of types between two species.

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## DISCUSSION

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AT present in the light of such data as is available from experimentation in the field of induced polyploidy with ornamental plants, we can not predict with any degree of assurance what practical results may emanate from this phase of genetics. It is generally acknowledged that many of our plants which are of horticultural importance are polyploid in nature and have arisen as natural hybrids or by selective breeding. However, we should

remember that many of our most useful crops are plants which are diploids.

What, may we ask, are the qualifications of a plant that make it a better or more desirable variety? Certainly it is neither because of its polyploid nature nor the size of the plant or flowers that makes the new form better fitted to survive environmental conditions. Plant breeders in their desire to create larger plants and larger flowers have been prone to overlook the fact that their new varieties are often less fitted to survive the variable cultural conditions under which they are to be grown than are the less desirable forms from which the hybrids were derived.

Those who are interested in genetics should strive toward a practical goal so that the application of polyploidy will be directed toward production of varieties of plants which have strong constitutions and are adaptable to many environmental conditions. There are several other improvements in plants which might be attained by the application of induced polyploidy in plant breeding: a wider range of season of bloom; development of fertile hybrids from sterile or less fertile types; winter hardiness and, lastly, the production of varieties less susceptible to diseases and insects.

To accomplish these aims it would seem quite desirable to emphasize the need of working intensively, either individually or cooperatively, with a few plants so that thorough observations can be made. The experimenter should endeavor to vary the concentration of colchicine solution used, the time of treatment and should consider the cultural temperature as well as the condition of the plant material.

Investigators should be cautious about selection of the most vigorous plants or seedlings and discarding apparently weaker or less significant material. For many times the least promising looking plants are ones which yield the desired results. This tendency on the part of the trained worker to select the more virile plants explains why the amateur with very little theoretical

knowledge often makes discoveries from the material the scientist would have discarded. Sterile or less fertile types of plants should not be promiscuously discarded but should be crossed or backcrossed to other genetic types of plants.

A new polyploid type of plant—once it is developed—should be cautiously introduced for commercial use until the reproductive ability and fitness of the plant to survive have been determined. The new plants should be distinctive and have characteristics that make them more valuable than existing forms.

# POLYPLOIDY AND MUTATIONS

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POLYPLOIDY has attracted interest from three somewhat distinct groups of workers on account of: (a) its significance in evolution, (b) its importance in plant breeding, and (c) its special chromosome mechanisms, with their significance for the elucidation of general cytological problems. Any discussion of mutation in polyploids must include all these aspects if we use the term mutation in its wider definition to include all classes of discontinuous change in the hereditary materials of the nucleus.

The evolutionary aspects of polyploidy received particular attention in the decade following Winge's (1917) survey of chromosome numbers in the plant kingdom and his theory of the origin of allopolyploids. Its importance for plant breeding became widely recognized after about 1921, and almost innumerable studies, particularly in the cereal crops, were carried out in the decade 1920–30. It is probably to Belling (1929) and Newton (see Newton and Darlington, 1929) that chief credit should go for first recognizing the value of polyploids for the elucidation of general problems of chromosome pairing, chiasma formation and crossing-over.

After about 1930 interest in polyploidy waned somewhat. It became realized that its significance in evolution is more limited than was at first thought and the cytological problems in which it proved particularly useful were mostly solved. For plant breeders it remained a most important tool but one that was not available at will—chromosome doubling had to depend on chance happenings which could at best be increased in frequency by wounding or decapitation, by heat or cold shocks, or by non-specific chemical treatments. The relatively recent discovery that colchicine has a specific action on the spindle which causes chromosome doubling in both ani-

mal and plant cells, and that it can readily be used to produce polyploids in a wide range of plants reawakened the earlier intense interest in their importance for plant breeding.

This brief outline is by no means intended as a review of the development of our present knowledge of polyploids. It *is* intended as an orientation and as a reminder that many of the problems now engaging so much attention were widely studied and in many cases solved a long time ago—as time is measured in the mushroom-like growth of cytogenetics. In preparing this paper I could find all too little of direct interest to plant breeders on mutation in polyploids to add to what has already been said by Stadler (1929, 1932), myself (Huskins, 1929) and many others a decade or more ago. Likewise little can be added on the cytology of polyploids to the review in Darlington's first edition (1932). With regard to evolution, however, a very distinct recent contribution has been made by Stebbins (1940), and Müntzing (1935) has shown that autoploidy has been more important than was formerly thought. There have also been great changes in general opinion on the nature of mutation and of the gene.

It seemed, therefore, that instead of merely presenting once again the data on mutation in polyploids, a more useful purpose might be served on the present occasion by concentrating on the definition and clarification of the aspects in which polyploids may be expected to differ most from diploids, even though in so doing we must skirt around the fundamental problem of the nature of the gene and adopt for present purposes a merely pragmatic grouping of the different kinds of mutation. Some confusion has undoubtedly arisen, particularly in the work of certain plant breeders, from failure to recognize fully the ways in which the genetics of polyploids, and the effects of mutations in them may be expected to differ from those of the materials of "classical" genetics or Mendelism. The exaggeration of the differences which I shall now attempt will, I hope, arouse thought and discussion and so serve to clarify the situation.

First, in polyploids we must perforce, at least for a long time to come, adopt the widest definition of mutation. It is to-day evident that even in Drosophila it is difficult to delimit gene mutations from chromosome aberrations —assuming as I do that the former really exist. In polyploids the distinction is virtually impossible. In passing I would comment that Stadler's (1932) conclusions from irradiation experiments on cereals anticipate some of the very recent discussions on the nature of mutations induced by x-rays. He was very dubious of the validity of the conclusion then commonly held that an appreciable proportion of them are chemical transformations.

Somewhat paradoxically, it is in cultivated plants, many of which are polyploids, that there exists some of the strongest evidence of a general nature for the reality and significance of gene mutation in the sense of chemical transformation—that is, for the concept that the hereditary material consists of discrete particles which can change qualitatively and so give rise to new characteristics that may have a chance to co-exist with or replace the original type. The mutations of Drosophila, on which our concept of gene mutation is so largely based, very rarely indeed exist in the homozygous state in nature. By contrast, in plants, both wild and cultivated, many alternative characteristics occur which are of equal viability and of which neither can with certainty be classed as the "wild type" or the mutant. They segregate as units at definite loci, so far as can be ascertained, though in no plant are they as adequately localized as in Drosophila. These are the kinds of changes implied in the original concept of gene mutation and believed by most geneticists to be the essential building blocks of evolution. They occur likewise in different races and species of animals, but sterility barriers are usually more effective in preventing their analysis in animals than in plants.

But in polyploids few if any mutations either "spontaneous" or induced, have yet been proved to be gene mutations, even in the limited sense of being changes

confined to single loci. In cultivated oats and wheat, mutations affecting chlorophyll development, glume color, glume shape, presence or absence of awns, glossiness of foliage, shape of panicle or spike, height, tillering capacity, time of heading and of flowering, failure of meiotic pairing and many other characteristics have almost all been shown to be due to chromosome aberrations affecting appreciable regions of or even whole chromosomes. The A Series fatuoid mutations of oats have not yet been definitely proved to be aberrations, but Series B and C fatuoids have gross chromosome differences and the analogous A Series speltoid mutations of wheat have been shown genetically, though not cytologically, to have an appreciable chromosome segment affected. In many of these mutants the progeny ratios are abnormal and in themselves indicate that the changes are not gene mutations. But in other cases chromosome aberrations give nearly normal Mendelian segregation ratios. Deviations in segregation ratios are almost exclusively due to male gametic inviability or competition; zygotic elimination plays only a minor rôle. The situation is very different in Drosophila where even minute deficiencies or duplications almost invariably seriously affect zygotic viability. It is different also in barley, which is a diploid. In it numerous spontaneous and induced mutations are known that fit the definition of gene mutations insofar as they are strictly localized changes, and many of them are completely recessive in their expression and without appreciable effect on either gametic or zygotic viability.

It is probable, of course, that as more mutations of polyploids are studied more will be found that are not associated with visible or genetically detectable changes in chromosome structure or number. In Drosophila, though many changes formerly classified as gene mutations are now known to be small deficiencies, duplications, inversions, or translocations, others have no structural basis visible even in the giant chromosomes of the salivary glands with their numerous distinctive bands.

In maize many mutations have no structural basis visible in the pachytene chromosomes but it must be remembered that in no plant can so detailed an analysis be made as in the salivary gland chromosomes of Drosophila.

The distinctions here expressed or implied between gene mutations and chromosome aberrations are, it must be emphasized, arbitrary ones. At the present time when opinions on the nature both of the gene and of gene mutation are in a state of flux, it would be foolish to attempt any precise definition of these terms. Suffice it to point out that in the past twenty years and particularly during the past six years since the development of salivary gland studies, the wheel of opinion has turned full cycle. Before that time, gene mutations were taken for granted by the majority of geneticists and it was the case for chromosome aberrations that had to be proved. Today, gene mutations are, as a first approximation, that residual class which remains after all attempts to demonstrate a physical basis for the change have failed.

It is, of course, most unlikely that the apparent rarity of gene mutations in polyploids indicates any real difference between them and diploids in the mutation process. Rather, the difference is almost certainly one of frequency with which the two types of mutation are able to survive or to be detected in diploids and polyploids.

The second problem we must consider is, then, that of distinguishing the frequency of occurrence of mutations of any kind from the frequency with which they make their visible appearance as mutant phenotypes. In diploid plants, on the one hand, chromosome aberrations will rarely survive through the haploid, gametophytic phase. Elimination of aberrant male gametophytes will be particularly severe. Mutant diploid zygotes will therefore be predominantly the products of gene mutation, or at least of very small aberrations and only rarely of chromosome aberrations large enough to be seen under the ordinary microscope.

On the other hand, in polyploids there is always a

strong possibility that the loss of chromosome parts or even of a whole chromosome will not greatly reduce gametic viability (or functioning provided there is little pollen-grain competition) since there are present other chromosomes that are "homoeologous" (Huskins, 1931), that is, homologous in large parts of their length, and which may therefore be able to fulfil the function of the missing parts. This applies even though their replicated sets of chromosomes may have become significantly differentiated both before and after the time when they became members of one complement. Chromosome aberrations may therefore readily survive in polyploids and some of them may be transmitted, particularly by female gametes, almost as regularly as gene mutations.

Apart from this difference in the survival rate of aberrations is the failure of gene mutations to find phenotypic expression in polyploids. Before a recessive gene mutation can affect the phenotype of a polyploid, that same gene must be lost or must mutate in a similar way in each of the replicated sets of chromosomes. This was clearly shown in the early work of Nilsson-Ehle on kernel color in wheat and of Akerman on glume color in oats, for which there exist three polymeric factors, and it was put to practical use by the latter in the production of a variety of black oats which throws far fewer white-grained mutant forms than the ordinary black oats of Sweden. Though polymeric factors can, of course, arise by segmental duplication and perhaps also by parallel mutation of non-homologous genes, their frequent occurrence in any species is a good indication of its polyploid nature. It may also be an indication that the polyploid species has existed for a long enough period of time to have permitted the occurrence of parallel mutations in all of the homologues of a given gene present in its replicated sets of chromosomes, but the homologous genes in question might, of course, have mutated in a similar direction before they came together in the allopolyploid. Hence gene mutations might show up in an allopolyploid

soon after its formation if a different set of chromosomes in each of its parents were differentiated in the same way.

As Stadler (1932) points out, polyploidy will not prevent the expression of gene mutations which have dominant expression. Now, the dominant mutations of Drosophila have been found to be predominantly duplications or deficiencies. The same applies to all those of polyploid cereals yet investigated. But the issue has been confused by different usage of the terms dominant and recessive. In Drosophila a gene which shows its effect in the heterozygote is classed as dominant or semidominant. In cereal genetics such mutants as fatuoids or speltoids are called recessives, although it is only in a few varieties that the heterozygote (which in Series B is a monosomic) can not very easily be distinguished from the "normal." It can always be distinguished from the homozygous mutant; hence it is more recessive than dominant, but by Drosophila standards it is a "semidominant." This may be part of the reason for the more frequent detection of speltoids and fatuoids than of any other cereal mutations. Sears (1940) has found a number of other monosomics to be almost completely recessive. The significance of this for the general problem of mutation frequency in polyploids relative to that of diploids is obvious.

A third major difference between diploids and polyploids is in the capacity of the latter to survive with homozygous deficiencies of one or more chromosomes. For instance, dwarf wheat plants of fair viability can survive with twenty pairs of chromosomes instead of twenty-one (Nishiyama, 1928, and many cases since).

A fourth difference is the ability of polyploids to compensate for the loss of certain chromosomes by the duplication of others. Wheat or oat plants entirely lacking the C chromosomes which are very important for the maintenance of vigor, fertility and normal meiosis can be almost normal in these respects if they have the deficiency made up by the addition of other chromosomes

which give no evidence from their structure or behavior of being even homoeologous (Huskins, Smith, Sander, Love, mss. in preparation). This situation seems to be entirely without parallel in diploids; the possibility that the extra chromosomes are homoeologous but so differentiated that they show no sign of their homology yet retain some of their original physiological functions can not, of course, be ruled out.

Related to the problem of the nature of mutations in polyploids is that of character expression. The difference from diploids is again only one of degree but it is so marked as to warrant distinct recognition. In all organisms a character is most probably always determined by the interaction of many genes; in polyploids this is so to the extent that we often can have no idea of the location or nature of the genes primarily determining the characters we are studying. For instance, the "speltoid complex" of genes in hexaploid wheat has, in ordinary genetic terminology, been shown to comprise at least two factors which are situated twenty-eight crossover units apart. One of these factors determines the presence of beards, the other affects the shape of the glumes. The two characters comprise the complete speltoid phenotype. In studies of speltoids with the normal chromosome number the only logical conclusion would be that the mutant genes as well as their normal "alleles" are on one particular pair of chromosomes. Yet forty-chromosome speltoids exist in which this pair of chromosomes is lacking. Actually the linkage has been measured only between genes which suppress the effects of the speltoid genes; the location of the latter is entirely unknown. They may originally have been alleles both in function and position, but now their relative positions are unknown, their common origin from two or more original genes is a matter of conjecture and only their functional aspect is determined. The description of the "normal" beardless and the mutant bearded genes of wheat as alleles, or, to take another example, the glume-shape

genes of normal *T. vulgare, T. spelta* and *T. turgidum* or the speltoid as a series of alleles K, $K_s$ and k is, on the original definition of an allelomorph (Bateson, see Darlington, 1932), to imply more than can be determined. They are functionally like alleles but we know almost nothing of their relative positions on the chromosomes, or of their origin. Actually, of course, the concept of allelism has altered in the course of the development of genetics but without, as far as I am aware, any clear statement of this having been made. The old definition in which position is stressed is still specifically stated or implied in even the most recent text-books.

If mutations in polyploids are predominantly due to chromosome aberrations we may expect a difference in the extent to which different agencies are responsible for their origin. Despite the enormous amount of work done on the effect of various radiations, all too little is yet known about the cause of gene mutation if we include in the definition of this term the concept that the changes are chemical transformations such as must surely have been involved in evolution. But many causes of chromosome aberration are known. It may occur "spontaneously" in either somatic or meiotic divisions. Heat or cold shocks cause many aberrations. X-rays produce them in quantity as do various chemicals. There are many "gene mutations" as well as deficiencies that cause asynapsis, polymitosis, "sticky" chromosomes and other abnormalities of nuclear or cell division which in turn produce various aberrations. And aberrations such as inversions or translocations in turn give rise to further aberrations, univalent chromosomes not infrequently fracture at the centromere and give "telocentric" fragments and "secondaries" or "isochromosomes," and so on.

The numerous reports in the older literature of mutations being produced after sudden frosts or periods of drought, or occurring more commonly in winter-sown than in spring-sown varieties of wheat or oats and so on

must therefore be taken more seriously than they were in the period when the term mutation was generally taken to connote gene mutation. A particularly clear illustration of the rôle of climate in producing mutations occurs in Trillium. *T. erectum* normally undergoes meiosis at temperatures little above freezing. If, however, the pollen mother-cells actually freeze during the process—this of course requires a severe lowering of the external temperature—divisions cease and then resume on thawing. They appear to be quite normal during the continuance of the meiotic divisions, but in the gametophyte divisions the chromosomes are often fragmented and completely abnormal. This seems obviously to be correlated with the high frequency of phenotypic abnormality so often reported in this species and with the cytological aberrations we have found in some collections of corms. *T. erectum,* it should be mentioned, is a diploid, but it reproduces in part vegetatively and the aberrations may thus be perpetuated instead of being eliminated as they would be in a diploid reproducing entirely sexually. In a polyploid reproducing entirely sexually many of them would be perpetuated through the protective action of replicated chromosomes, as are the aberrations causing speltoids and fatuoids.

Perhaps the most important cause of aberrations in polyploids is hybridization. The over-simplified and over-emphasized view of the rôle of hybridization in causing variation which has been propounded by Jeffrey and others has probably been partly responsible for underestimation of its significance by the majority of geneticists. Hexaploid wheat and oats are normally self-fertilized and hence homozygous, with very regular meiotic divisions in spite of their polyploid nature. But whenever crosses occur, even between very similar varieties, the hybrids are apt to be characterized by abnormalities such as multivalent and univalent formation, and the occurrence of bridge chromosomes at anaphase. The latter are, of course, an indication of relative inversion

of chromosome segments. These irregularities of meiosis produce a range of aberrant types of progeny, many of which are of normal or nearly normal zygotic viability, though if they are heterozygous there is usually gametic elimination through pollen-grain competition. It has become evident from our studies of mutations in wheat and oats that a large proportion of them owe their origin thus indirectly to the occasional natural crossing that occurs in these ordinarily self-fertilized species. Further, many of the mutant forms thus produced are, on account of the reduced functioning capacity or partial sterility of their pollen, far more apt to be cross-fertilized than are normal plants of the same variety. Hence, again, aberrations induced by natural crossing, lead to further aberrations through increased tendency for outcrossing. The same must apply of course in part to diploids but the features we have considered previously are responsible for the greater survival rate of aberrations in polyploids. As an added factor note Sturtevant's (1939) observation that hybridization increases the mutation rate (in Drosophila).

Since "haploid" plants from polyploid species have two or more sets of chromosomes, surprise has often been expressed that they are usually little if any more vigorous than true haploids from diploid species. Both this and the rarity of haploids in animals other than those with the haplo-diploid mechanism of sex-determination are, however, expected on the basis of the differences in time, place and extent of elimination of chromosome aberrations outlined above—though naturally it is not assumed that in these lie the whole explanation. Again, of course, the differences are only of degree and not absolute. They may be expressed, roughly, as follows: In an animal with a haplo-diploid sex mechanism there will be rigid selection against abnormalities in a set of chromosomes every time it comprises the genetic constitution of a male animal. It will therefore be expected to remain "normal" or self-sufficient over long periods of evolu-

tionary time. In a diploid plant there is rigorous elimination of defective chromosome sets during the male gametophytic stage. The survival of some defective female gametophytes, presumably because they are nourished by the parent zygote, prevents the elimination of defective sets being as complete as in haplo-diploid animals. It is, however, more effective than in ordinary diploid animals, in which there are, of course, no mitotic divisions following meiosis and little if any elimination of gametes carrying chromosome aberrations. Elimination occurs almost exclusively in the zygotic stage of diploid animals; aberrations in one set of chromosomes can therefore be perpetuated if they are protected by compensating factors in the other set. In the course of time a single set of chromosomes may, then, be expected to become insufficient for maintaining existence. In polyploid plants there is only moderately effective elimination of male gametophytes defective in only one of their replicated sets of chromosomes and almost none of female gametophytes. A defect in one set can be compensated by factors in either a homologous or a homoeologous chromosome. The "haploid" complement of a polyploid may therefore in the course of time be expected to acquire so many defects that it is little if any more self-sufficient than that of a diploid. While this argument consciously ignores differences between plants and animals in complexity of organization and physiological functions, and is obviously an incomplete analysis in other respects also, it does seem to provide one reasonable basis for the observed facts of haploidy.

Two points of more immediate significance for plant breeders may next be considered. The pure-line theory is at best a limiting concept; cytological study of polyploids makes it evident that in them it has particularly limited validity. Polyploids can breed true only so long as pairing is rigidly restricted to chromosomes that are completely homologous. Any pairing, even of small segments, between homoeologues will, if chiasma formation

occurs, undo the homozygosis that has been brought about by generations of self-fertilization.

Occasional irregularities of pairing occur at meiosis even in old-established varieties of wheat or oats, though much less frequently than might perhaps be expected from their hexaploid nature; in varieties of recent hybrid origin irregularities are very frequent (see Love, 1940, and earlier). In Sorghum, Huskins and Smith (1934) found multiple chromosome associations that would surely cause abnormal segregation or "chromosome mutation." These caused them to doubt that the grain Sorghums and, by analogy, Maize are true diploids as commonly assumed. If they are not, then even though from the plant breeder's point of view they may behave almost completely like diploids, their genetic constancy and the manifestations of hybrid vigor may be affected in ways distinct from those in true diploids. Again, of course, we encounter the fact that no really sharp line can be drawn between diploids and polyploids any more than between auto- and allopolyploids. In experimentally produced polyploids there is every gradation between the complete autopolyploidy which results from somatic chromosome doubling in a homozygous diploid and the extreme degree of allopolyploidy or amphidiploidy found, for instance, in Raphenobrassica, the fertile tetraploid derivative of a cabbage-radish cross (Karpetchenko, 1927). On the other hand, duplications may become established in species with a basic diploid chromosome number. It is not therefore surprising that it is often difficult to determine whether some long-established species should more properly be considered diploids or polyploids. Maize and Sorghum are outstanding examples. When the argument was first advanced (Huskins, 1929) that the pure line theory lacks rigid validity for polyploids, it was based almost entirely on *a priori* expectations from cytological observation. It has, however, since been supported by the genetic observations of many plant breeders and the necessity for a

close cytological check-up of all types of breeding work is now widely recognized.

The phenomenon of "shift" (see Sansome and Philp, 1939) which is found in crosses between polyploid species has also been plausibly, and almost surely correctly, supposed by Darlington (1927) to be due to autosyndesis, or, in the terminology above, to pairing between homeologous chromosomes.[1] If so, it is of course a phenomenon confined to polyploids, with the probably insignificant exception that it might occur in diploids with reduplicated segments.

In conclusion we may compare the points of view expressed above, which have been reached in the course of extensive studies of mutations in cereals, with the observations and opinions, based on cyto-systematic studies, of Stebbins (1940) on the rôle of polyploids in evolution. Of Crepis, Stebbins says: "Although there is much evidence to show that this complex has existed since the late Tertiary epoch, its polyploid members have not in all this time evolved any new characteristics." He states further: "The sexual polyploid complex of Paeonia seems, according to my present knowledge to hold to the same rule, in spite of the fact that it is obviously very old. In any case, however, the odds are against the polyploid complex as being the originator of any really new line of evolution. As compared with a group of diploid species, a polyploid complex tends to be a closed system. It can produce endless new species, but these are all or nearly

[1] The term "homoeologous" may be considered unnecessary in a subject already overburdened with synonymous terminologies, but it avoids both the circumlocution of "chromosomes that are homologous in parts of their length," and the etymologically unjustifiable "partially homologous," which is often used. Its use here has the further merit of clarity which is sometimes lacking in the use of the terms allo- and autosyndesis, as Waddington (1939, p. 73) apparently found. His references to "Philp and Huskins" in this connection should, however, as he has pointed out in a personal communication, all be to "Sansome and Philp" and it appears probable that he has also misinterpreted their meaning. Confusion is particularly easy when tetraploids newly arisen from crosses of diploid species are not sharply distinguished in the argument from tetraploids derived from crosses of tetraploids.

all new combinations of the same supply of genic material; they are new variations on an old theme." And again, according to Stebbins's evidence: "Since the polyploid members of a complex are more numerous and widespread than the diploids, one would naturally expect that as a polyploid complex becomes older and as conditions cease to be favorable for the type of plant represented by that particular complex, its diploid members would be the first to go. An old or senescent polyploid complex, therefore, is one that consists only of polyploids. With increasing age, the polyploids also begin to die out, so that in the last stages of its existence a polyploid complex is simple once more, and is a monotypic or ditypic genus without any close relatives."

These conclusions seem to be entirely in line with the *a priori* expectations from the views I have expressed above. In brief, the mutations of polyploids are chiefly chromosome aberrations which give rise to new combinations and new balances, but which are not likely to yield radically new characteristics. Qualitative changes which must surely be a vital part of the evolutionary process can probably occur equally frequently in diploids and polyploids, but will not so readily find expression in the latter and will not therefore as often be subjected to either positive or negative selection pressure. Their survival will be dependent almost entirely on chance; this may, however, operate to make them most important agencies of evolution on rare occasions. Obviously there can be no hard and fast lines of demarcation between diploids and polyploids in any of the respects considered. It may, however, have been of some value to draw attention to ways in which they may be expected to differ in degree.

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## DISCUSSION

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I HAVE chosen to limit these brief remarks to just one of the varied phases of the problem of mutation in polyploids that Dr. Huskins has touched upon this morning: that is, the relation of polyploidy to evolution. There can be little doubt that typical recessive gene mutations have much less opportunity to find expression in polyploids than in diploids. In autopolyploids, a tetraploid

for example, a recessive mutation is "covered" by three normal alleles, thus, AAAa. If the chromosomes are homologous and associate as quadrivalents, it is likely to require two generations of selfing (assuming the plant to be self-fertile) to get out the first quadruple recessive, aaaa. This is definitely more difficult than in a diploid, where the recessive character should appear in one fourth of the $F_1$ population after a mutation.

If the polyploid in question is an allopolyploid, that is, one that originated by chromosome doubling in a more or less sterile hybrid, the difficulty of a recessive mutation expressing itself is still further increased. Here the chromosomes from the two parents may be so differentiated that they synapse largely by autosyndesis, as bivalents: AA and Aa. Under these conditions there is no exchange of material between the bivalents, and segregation alone can not produce a quadruple recessive; therefore, a dominant allele from the other bivalent must be lost, or must mutate separately, for a quadruple recessive to result.

In actual practice it is usually difficult to differentiate sharply between these two types of polyploids, but in either case mutant characters will appear less frequently than in diploids. As Dr. Huskins has pointed out, gene changes that do not express themselves can not be subject to positive or negative selection pressures. Polyploids, therefore, once they have become polyploid, should not be expected to be of great importance in further evolution.

Of course, this should not obscure the immediate rôle of the polyploid in the plant scheme. A large portion of the higher plants have gametic chromosome numbers which are simple multiples of those found in related species, and as we have seen again this morning, many of our important domestic plants, including the best varieties of cotton, tobacco, oats, wheat, flowers, etc., are polyploids.

Before concluding, however, I should like to introduce

a bit of controversy into the discussion. There are other points of view which differ from that expressed above.

If Goldschmidt is correct in his "Material Basis of Evolution" gene mutations can no longer be looked upon as important in the larger aspects of evolution; these are important only in microevolution, or diversification within a species. Goldschmidt considers "systemic mutations"—gross rearrangements of chromosomal materials—responsible for macroevolution, or the separation of larger groups such as genus, family, order, etc. If this be true, perhaps the failure of recessive mutations to express themselves in polyploids is of less importance than we think.

Secondly, I should like to again call forth the thought that evolution must eventually require something more than mere *change* in genes—that it must continually require *new* genes in the building of plant and animal kingdoms. The possibility has been suggested by various workers that polyploidy may offer a means of securing such new genes. During the early life of both auto- and allopolyploids many genes are "covered" by duplicate genes. Gene mutation, both good and bad, and chromosomal aberrations of all types, including deficiencies and duplications, are largely free to go on in this protected condition without immediate danger of elimination by natural selection. Possibly this provides an opportunity for the plant to experiment with its evolutionary materials, especially in rearranging them. During the evolutionary history of a polyploid, then, changes are relatively free to proceed in perhaps as much as one half of the genic material. It is conceivable, though as yet not demonstrated, that duplicate genes might so differentiate during long periods of time as to take on new functions, to lose homology with the old genes, and to again become exposed to the directing influences of natural selection.

# AN EVALUATION OF INDUCED POLYPLOIDY AS A METHOD OF BREEDING CROP PLANTS

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THE present widespread activity in the experimental production of polyploid plants raises the question of the practical value of this type of experimental work as a method of plant breeding. During the past few years more than two hundred papers dealing with the scientific aspects of induced polyploidy have been published. The claim is being made that the much-publicized colchicine technic constitutes a new method of plant breeding capable of producing at will new species and new giant varieties of horticultural and crop plants. The implication is that these new forms at once constitute superior kinds of plants of inestimable practical value, wholly unlike anything the plant breeder has ever before produced. Others are contending that little or nothing of practical value is to be expected from the polyploidy technic. Under these circumstances it would seem to be desirable to attempt an evaluation of the results that have been obtained up to the present time. This symposium, organized as a forum discussion of theoretical and practical aspects of polyploidy in crop plants, is most timely and should serve to clarify research objectives in the general field of induced polyploidy.

Fortunately, induced polyploidy is not a new field of investigation, and it certainly does not constitute a new method of plant breeding. Experimental work on the effects of various physiological factors on chromosome and cell division was initiated at the beginning of the present century. The very extensive physiological experiments and cytological observations of Nemec, Lundegårdh and Sakamura, begun more than a quarter of a century ago, demonstrated that chromosome doub-

ling could be induced by subjecting dividing cells to various chemical and physical agents, including chloral hydrate, ether, chloroform, and rapid and extreme changes in temperature. New polyploid forms were first produced in 1908 by the Marchals in their classical regeneration experiments with mosses. Among the higher plants, tetraploid strains were first induced experimentally in various species of Solanum by Winkler in 1916. His graft hybrid technic has since been used with modifications by von Wettstein (1924), Jörgensen (1928), Lindstrom and Koos (1931), Greenleaf (1934), and others to induce chromosome doubling not only in solanaceous plants but also in various other kinds of plants that can be propagated vegetatively.

During the past decade tetraploidy has been induced in plants propagated by seeds by applying temperature shocks to the plant in the initial stages of embryogeny. This technic was first developed in experiments with maize (Randolph, 1932) and has since been used successfully in various cultivated plants (Dorsey, 1936; Atwood, 1936; Peto, 1936; Müntzing, *et al.*, 1937; Straub, 1938; Cooper, 1939; and others).

These and other methods of inducing polyploidy, together with the colchicine technic, provide a variety of procedures for doubling the chromosomes of cultivated plants. If one method fails, another can be tried. Polyploidy has already been induced in many different kinds of plants, and it is probably not an exaggeration to say that at the present time methods are available for producing polyploid strains of almost any plant that the experimentalist may wish to investigate.

Most of the results obtained with the colchicine technic are of too fragmentary a nature to be of much value in estimating the usefulness of induced polyploidy as a method of plant breeding. More reliable data are to be obtained from the experimentally induced polyploids whose breeding behavior has been studied over a period of years.

It will not be possible in the time at my disposal to survey the entire field of induced polyploidy in relation to the breeding of horticultural and other crop plants. The experimental work in this field includes, (*a*) the production of autopolyploids by direct chromosome doubling, and (*b*) the production of allopolyploids by chromosome doubling following hybridization. Both lines of investigation are producing results of very great theoretical and practical interest; but the former is perhaps of more immediate practical interest to the plant breeder. Since allopolyploidy is to be discussed at some length by other speakers in this symposium, my remarks will be limited chiefly to a discussion of the effects of direct chromosome doubling.

It is wishful thinking to assume that any and all plants can be improved by doubling their chromosomes. It should be borne in mind that many of our cultivated plants are already polyploids and further reduplication of their chromosomes is more often detrimental than beneficial. This fact is amply illustrated by the doubled forms of our common cereal grains produced experimentally by Dorsey (1936) and other workers. Rye, barley and *Avena brevis* are diploids, and vigorous, relatively fertile tetraploids have been obtained from them. Most cultivated varieties of wheat and oats on the other hand are natural hexaploids and their doubled forms are invariably dwarfed and deformed in various ways, and are also highly sterile. Similar results were obtained by Johnstone (1939) in experiments with the cultivated potato and various other species of Solanum. Commercial varieties of *Solanum tuberosum* are natural tetraploids. The doubled forms of the varieties Russet Rural and Golden, having the octaploid chromosome number, were dwarfed, succulent types appreciably less vigorous than the parent varieties. Furthermore, the tubers of these doubled potato varieties were reduced in size and the yield of tubers was low. The wild tetraploid species, *Solanum andigenum,* when doubled, was also less vigor-

ous than the typical form. However, tetraploids derived from the diploid species, *S. Jamesii, S. chacoensis* and *S. bulbocastanum,* were as vigorous or more vigorous than the typical species and were also quite fertile. Many strains of tetraploid maize are more vigorous than the parent diploids in that they are somewhat taller and more sturdy; but octoploid maize is invariably much less vigorous than the tetraploid and is completely sterile. Comparable results have been obtained in Nicotiana, Petunia, Gasteria, Chrysanthemum and other genera. It is quite evident that there is an upper limit, or threshold, beyond which further reduplication of chromosome sets produces markedly deleterious results. This threshold may be at the tetraploid, the hexaploid or even the octoploid level in different genera, and appears to be dependent, in some degree at least, on the extent to which hybridization has been involved in the evolution of the species or varieties in question.

Autotetraploids and other autopolyploids are referred to not infrequently as gigas or giant forms, following the examples of DeVries, whose *Oenothera gigas* was probably the first spontaneous autotetraploid mutant to be observed among cultivated plants, although it was not until some years later that its true nature was determined by chromosome count. It is now generally recognized, and in fact has been emphasized by East and others, that most autotetraploids are not giant forms at all. More often than not they are little if any larger in growth habit than the parental diploids. But they are typically sturdier and more robust, characteristics that may be of appreciable value to the plant breeder.

Of considerable importance from the practical standpoint is the fact that chromosome doubling ordinarily increases the size of the reproductive structures of the plant: flowers, fruits and seeds ordinarily are considerably enlarged. The seeds of doubled rye, barley, sudan grass, buckwheat and maize, for example, are from 25 to 75 per cent. larger than the seeds of the parent diploids. Such significant increases in seed size might be expected

to result in corresponding increases in yield. But this does not happen in the cereal grains nor in various other crop plants, because of a reduction in fertility that characterizes most autopolyploids.

This reduction in fertility may vary from only 5 or 10 per cent. to almost complete sterility. The sterility present in most autotetraploid crop plants thus far produced is sufficient to constitute a serious barrier to their practical utilization unless their fertility can be increased by breeding and selection. There is some evidence that hybrid combinations of unrelated autotetraploid stocks within a given variety are more fertile than the parent stocks, and this may provide a means of developing more fertile autotetraploid varieties.

Among the cereals most of the strains of tetraploid barley that have been produced by Dorsey and other workers are highly sterile, although one of Müntzing's strains and a spontaneous tetraploid mutant discovered by Harlan and Wiebe are more fertile. Doubled Rosen rye in field plantings exhibits varying fertility among individual plants ranging from very poorly filled heads to heads that are almost perfectly filled. An acre planting of doubled Rosen grown at Ithaca this past year produced a yield of 15 bushels. It was estimated that the average fertility of this planting did not exceed 60 per cent. The grain was much larger than that of ordinary diploid Rosen rye. The plants were more vigorous, tillered profusely and had a stiff upright growth habit markedly resistant to lodging. This strain of doubled Rosen came originally from three tetraploid plants produced by Dorsey from heat treatments in 1935. If the fertility of this strain could be increased somewhat, it would certainly outyield the parent diploid variety. An attempt to increase the fertility of doubled Rosen by selection is now in progress.

The variation in fertility that is characteristic of tetraploid maize has been studied in considerable detail in an effort to analyze the factors concerned. Tetraploidy has been induced in open-pollinated varieties, in inbred lines

and in various hybrid combinations of inbred lines. The inbreeding of hybrid tetraploid stocks, accompanied by the selection of more fertile and less fertile lines, has been practiced on an extensive scale over a period of years. Since modern corn breeding is based on the production of hybrids among inbred lines, the effects of inbreeding and cross-breeding tetraploid stocks have received special attention. Cytological and genetical analyses of the causes of the observed differences in self-fertility also have been undertaken.

Some of the more important results of these studies of tetraploid maize may be summarized as follows:

(1) Tetraploids produced from open-pollinated varieties and hybrid stocks are more highly self-fertile than those produced from inbred lines. Tetraploids derived from the heterozygous stocks are ordinarily from 70 to 90 per cent. fertile and are as vigorous or more vigorous than the parent stocks.

(2) Selection for increased fertility and vigor in open-pollinated tetraploid stocks originating from hybrid diploid plants has been practiced on a limited scale for the past eight years, and strains of vigorous sturdy plants with an average fertility of 70 to 80 per cent. have been developed. Extensive yield trials have not been conducted, but it is probable that the yield of the best open-pollinated tetraploid stock thus far produced is not significantly greater than that of the comparable diploid. This does not mean that it is impossible to produce superior strains of doubled maize. More trials are needed to determine this.

(3) Tetraploids produced directly from diploid inbred lines are ordinarily very highly sterile and are conspicuously lacking in uniformity and vigor. Because of these characteristics they are extremely difficult to maintain, and with but a single exception numerous attempts that have been made to produce tetraploid inbred stocks by the direct doubling of diploid inbred lines have failed.

(4) The inbreeding of a considerable number of hybrid tetraploid stocks for from 5 to 8 generations has resulted in the segregation of relatively fertile and highly sterile inbred lines. In the discussion that follows, these and others that are like them will be referred to simply as "fertile" and "sterile" lines. These inbred lines are not true breeding for a given level of fertility, but vary considerably with respect to the amount of self-fertility of individual ears. The seeds of partially filled ears are scattered uniformly over the ear, and there is no evidence of any significant variability with respect to fertility within individual plants.

(5) Hybrids between certain inbred tetraploid lines are highly fertile and exhibit pronounced hybrid vigor.

(6) Cytological studies of tetraploid maize have shown that open-pollinated fertile, inbred fertile and inbred sterile tetraploid lines maintained for from 5 to 8 generations since they were originally produced exhibit the same variation in chromosome number that characterized the original parental

tetraploid plants. It was observed by Kadam that in any given population approximately one half of the plants have the balanced chromosome number and the remainder have one or a few chromosomes more or less than the balanced number.

(7) Meiotic chromosomal irregularities are no more prevalent in the sterile lines than they are in the fertile lines. The mean number of quadrivalents formed in both is essentially the same and ranges from 7.5 to 8, according to the observations of Kadam and Fischer.

(8) Abundant good pollen is ordinarily produced by both sterile and fertile tetraploid stocks.

(9) It is concluded from the cytological analyses of the fertile and sterile tetraploid stocks that chromosomal irregularities in meiosis probably account for reductions in fertility of from 5 to 15 per cent., but the higher amounts of self-sterility must be due to other causes.

(10) Genetic analyses of self-sterility in tetraploid maize, made by Dr. Harold Fischer, indicate that self-fertility is partially or completely dominant to self-sterility in the $F_1$, and back-cross and $F_2$ data suggest that segregating sterility factors may be responsible for the observed differences in degree of self-fertility.

(11) The partial self-sterility of tetraploid maize is due exclusively to conditions associated with the doubled number of chromosomes and can not be attributed to other heritable changes induced by the heat treatments with which the tetraploids were produced. Evidence in support of this conclusion has been obtained from parthenogenetic diploids that occur spontaneously in tetraploid maize with a frequency of about one per one thousand. These parthenogenetic or maternal diploids, except for occasional unbalanced aneuploid chromosome types, are completely fertile whether they arise from the fertile or from the sterile lines.

(12) Self-sterile tetraploid maize differs from the type of self-sterility that characterizes diploids of the Nicotiana type in that most stocks are partially fertile and considerable variability in the amount of sterility is prevalent within individual inbred lines, even after as many as 8 generations of inbreeding by selfing individual plants. Furthermore, in the various intercrosses of different self-sterile lines or of self-sterile and self-fertile lines that have been made thus far, the self-sterile lines ordinarily are cross-sterile when used as the seed parent, but in reciprocal crosses with self-fertile lines they may be cross-fertile. There is also some evidence of cross-incompatibilities between certain self-fertile stocks. However, this entire subject needs further study. In the Nicotiana type complete self- and cross-sterility or fertility is the rule, depending on the genotype involved; only under very special conditions are self-sterile types at all self-fertile.

The reduced fertility in autotetraploids has been attributed by Darlington (1932, 1937), Kostoff (1939), and others to meiotic chromosome irregularities resulting from the multivalent association of the chromosomes in synapsis. It is argued that autotetraploids with small chromosomes have fewer chiasmata and therefore form

fewer multivalents, and for this reason are more fertile than similar forms with larger chromosomes and more multivalents. This view has not been accepted by Müntzing (1936), who cites examples of autotetraploids with small chromosomes and bivalent synapsis that are quite sterile, notably in Solanum, and further emphasizes that many spontaneous autotetraploid species and races in nature are highly fertile, although multivalent associations are present. Müntzing attributes the sterility of autopolyploids chiefly to physiological factors of undetermined nature.

Obviously, the hypothesis of Darlington and Kostoff is inadequate as an explanation of the range of variation in fertility that is found in tetraploid maize, especially as it has been shown that the highly fertile and highly sterile stocks have essentially the same chromosome number relations and the same synaptic behavior in meiosis.

An adequate explanation of the variable fertility in maize is not apparent at the present time, but it has been quite definitely established that the immediate cause is not the lack of sufficient viable pollen. Most tetraploid stocks shed abundant good pollen and in making pollinations adequate precautions are taken to make sure that an excess of fresh pollen reaches the silks while they are fully receptive. A detailed cytological study of the fertilization process and the subsequent events in both sterile and fertile lines is now in progress.

Of major importance to the plant breeder is the fact that some autotetraploid stocks are more fertile than others. Thus it should be possible by selection to increase fertility. This has already been accomplished in tetraploid maize. Fertile inbred lines have been produced, and hybrids between different lines are vigorous and fertile. Complete fertility probably can not be attained in autotetraploid maize or in the doubled forms of most other cultivated plants even by the most rigid and prolonged selection because of inherent chromosomal irregularities. But this does not necessarily mean that superior

strains of autotetraploids can not be produced. Autotetraploids may have advantages such as increased seed size, increased nutritional value, improved growth habit, etc., that more than compensate for a partial reduction in fertility.

In connection with the sterility problem in autopolyploids, it is encouraging to note that the autotetraploid species and races occurring in nature, that have been reported in increasing numbers in recent years, are in general much more fertile than most experimentally induced autopolyploids. Müntzing has emphasized this fact and has attributed the difference to natural selection that has operated in nature to increase the fertility of the wild autotetraploid forms.

Attention has been focused on the reduction in fertility that characterizes most experimental autopolyploids, as it is probably the most important limiting factor in their practical utilization. But as previously stated superiority in other characteristics may outweigh the deleterious effects of reduced fertility.

It is becoming increasingly apparent from the researches of the past few years that chromosome doubling produces not only morphological alterations but profound physiological and chemical changes as well. Differences in osmotic pressure that may influence resistance to frost injury and winter hardiness have been reported. According to Becker (1931), osmotic concentration in autopolyploid mosses is inversely proportional to chromosome number. Several workers have determined the water content of diploid and autotetraploid tomatoes. Some have reported increases in certain doubled strains, while others found no significant differences in the strains they examined. A decrease in water content of from one to two per cent. and a corresponding increase in dry matter was reported in a doubled strain of *Nicotiana Tabacum* by Noguti, Oka and Ôtuba (1940). However, the doubled strain of tobacco wilted more readily in bright sunlight than did the ordinary tobacco, indicating

that it had either a higher water requirement or increased permeability. It is also possible that transpiration was more rapid in the tetraploid due to the increased size of the stomata, which ordinarily results from chromosome doubling, provided there was not a compensating decrease in the number of stomata per unit area.

From observations of tetraploid maize grown under field conditions during the past eight years, it is apparent that there is little if any difference between the diploid and tetraploid with respect to drought resistance, resistance to frost injury or in resistance to the common diseases of maize. However, most strains of tetraploid maize, in common with numerous other autotetraploids, mature somewhat more slowly than the related diploids: the pollinating season is attained several days later and the grain needs a somewhat longer growing season to reach maturity.

Extensive data on the chemical composition of comparable diploids and tetraploids are not available, but a beginning has been made in this important field of investigation. Increases in the vitamin C content of fruits and vegetables as a result of chromosome doubling have been reported. Tetraploid yellow corn meal has approximately 40 per cent. more vitamin A activity than the original diploid, according to the analyses of Randolph and Hand (1940). Increases in the nicotine content of tobacco ranging from 18 to 33 per cent. were reported in the doubled varieties analyzed by Noguti, Oka and Ôtuba. But the yield of these same tetraploid varieties based on the dry weight of the leaves was reduced more than 50 per cent. These same workers also reported that significant increases in total nitrogen, fats, calcium, potassium and magnesium and compensating decreases in carbohydrates, sulfur and phosphorus resulted from chromosome doubling in cultivated varieties of tobacco. Chemical analyses of diploid and tetraploid clones of perennial rye grass, *Lolium perenne*, derived from the same original colchicine-treated seedlings, were made by Sullivan and Smith (1939). These analyses indicated that the tetra-

ploids had a significantly higher sugar content than the diploids and relatively more dry matter, but that they did not differ significantly in total nitrogen.

A very interesting comparison of yield and sugar content in diploid and triploid sugar beets was published recently by Peto and Boyes (1940). The triploids were definitely superior to the diploid, the mean increase for root weight being 12.2 per cent., for yield of sugar 14.9 per cent. and for dry weight of the tops 17.8 per cent. Particularly significant was the fact that the percentage of sugar did not decrease with increase in root size as rapidly in the triploid as it did in the diploid. If these findings are confirmed by more extensive trials, polyploidy may be of considerable value as a method of increasing yields of sugar in the sugar beet and possibly also in other sugar-producing plants, although most commercial sugar-cane varieties are already highly polyploid and further increases in chromosome number may be detrimental.

Analyses of the chemical composition of tetraploid maize undertaken at Ithaca in collaboration with the U. S. Nutrition Laboratory indicate that in certain stocks of maize chromosome doubling increases the protein content of the corn meal from 5 to 20 per cent. The amount of the increases differed for different stocks.

From these and other physiological and chemical analyses of induced polyploids, it may be concluded that some of the important characteristics of at least some of our cultivated plants can be improved by chromosome doubling. However, it should be borne in mind that percentage increases of certain constituents entail compensating decreases of others. If important constituents, such as the protein content of cereals, the vitamin content of fruits and leafy vegetables and the sugar content of sugar-producing plants are increased significantly by chromosome doubling without attendant deleterious effects, induced polyploidy will most certainly become an increasingly important method of plant breeding. It might be mentioned in passing that increases in the various constituents of cultivated plants may not always be

desired. For example, some forage plants contain appreciable amounts of alkaloids and other toxic substances which it would be desirable to decrease rather than increase, and for certain uses it would be advantageous to decrease the amount of nicotine in commercial varieties of tobacco.

At the present time, additional analyses of strictly comparable diploids and tetraploids are urgently needed to clarify the present rather confused situation relative to the physiological and chemical effects of chromosome doubling. Apparently, only certain characteristics are affected in a given plant, and these specific effects may differ in different plants, both in degree and in the nature of the effects produced.

Now this is exactly what would be expected if these new characteristics exhibited by autopolyploids were regulated by specific genes acting in a cumulative manner, rather than being brought about by the doubling of the chromosomes per se. Such an interpretation was advanced to explain the increase in the carotenoid content of tetraploid maize observed by Randolph and Hand. In this case, there was a 40 per cent. increase in the carotenoid content of the tetraploid, even though there was a greater concentration of genes per unit volume in the diploid than in the tetraploid, due to an increase of 3.5 times in the volume of the endosperm cells of the tetraploid. Also, the amount of carotenoid elaborated per gene was 2.5 as great in the comparable cells of the diploid. The relations between cell volume, gene concentration and the relative amounts of such products of cell metabolism as proteins, fats and carbohydrates have not been determined in most instances in which various differences between diploids and their autopolyploid derivatives have been analyzed, but an interpretation of the available data based on the assumption of specific gene action seems to afford a relatively simple explanation of the known facts. Differences in the kinds of genes comprising the genotype probably explain the different

effects produced by chromosome doubling in different species and in different strains within a given species.

A similar interpretation may explain many of the diverse morphological effects encountered among autopolyploids. This subject will not be discussed in detail here, but it is quite obvious the situation is not as simple as most workers have assumed. An increase in cell size roughly equivalent to the increase in nuclear volume that results from chromosome doubling is a common occurrence in autopolyploids, and there often appears to be a direct causal relation between increased cell size and the enlargement of such structures as pollen grains, stomates and root and stem meristem. But not all organs are similarly affected, especially the more complex ones. Pronounced shape differences are also prevalent. Ontogenetic studies reveal differences in the rate of cell division and in the amount of cell enlargement that take place as differentiation proceeds. Such differences influence directly the size and shape of the fully differentiated organ. For example, the pollen, epidermal cells, stomates and the cells of the stem meristem of tetraploid maize are approximately twice as large as those of the ordinary diploid, but the cells comprising the endosperm tissue at maturity are 3.5 times as large as those of the diploid, at least in some strains. Since the endosperm tissue of the tetraploid is rarely more than 50 per cent. larger than that of the diploid, it must contain less than half as many cells as the diploid. Thus the increase in the size of the endosperm tissue as a whole has been accomplished by an almost prodigious cell enlargement accompanied by a marked reduction in cell division activity. Obviously, chromosome doubling does not always affect the cellular organization of the various tissues and organs in a similar manner.

From the viewpoint of the plant breeder who is interested in the production of superior varieties of cultivated plants, it is extremely important to know not only what are the immediate morphological and physiological

effects of chromosome doubling, but also the manner of inheritance of any new characteristics that may be produced. In the present state of our knowledge, it must be admitted that the effects of chromosome doubling for a particular species or variety are largely unpredictable, and the available information on which to base predictions of the breeding behavior of new characters thus produced is very limited.

The genetics of autotetraploids have been studied sufficiently to indicate that tetrasomic inheritance, rather than the disomic type of inheritance that characterizes diploid organisms, prevails very generally as would be expected since four sets of homologous chromosomes instead of two are normally present. The consequences of this are of far-reaching importance to the plant breeder. For example, a much longer period will be required to fix a given character, and inbreeding programs designed to obtain stock for inter-crossing to give hybrid vigor must be appreciably extended. Conversely, autopolyploids have certain practical advantages. Since there is less rapid segregation of recessive deleterious characters, stocks will tend to remain more uniform for longer periods of time. Theoretically, heterosis should also persist at a higher level in subsequent generations after the original cross is made. If this should happen in actual practice, the farmer who grows tetraploid hybrid corn could profitably save his own seed for at least a year or two and would not have to purchase new hybrid seed each year as he does when growing ordinary diploid corn. An advantage such as this would be a matter of very great practical importance.

Mention should also be made of certain other characteristics of autotetraploids that are of primary importance from the practical breeding standpoint. Autopolyploids once produced tend to breed true for the doubled condition of their chromosomes, except for deviations in number of one or a very few chromosomes that apparently do not affect the maintenance of the tetraploid state

in succeeding generations. However, diploid individuals do appear at rare intervals in the progenies of some autotetraploids as a result of the parthenogenetic development of their normally diploid female gametes. In maize these parthenogenetic or maternal diploids occur with a frequency of about one per thousand (Randolph and Fischer, 1939). This means that in an acre of tetraploid maize about a dozen diploids may be expected. These derived diploids are completely fertile, but their occurrence is not a serious matter in naturally cross-pollinated plants such as maize since diploids and tetraploids are ordinarily very highly cross-sterile. Triploids which might be produced occasionally from natural crossing of the diploids and the parental tetraploids are relatively infertile when intercrossed or when crossed with tetraploids. But among naturally self-pollinated plants, these parthenogenetic diploids would be expected to perpetuate themselves and appear as "contaminations" of the original tetraploid strain in subsequent generations.

Little is known about the prevalence of parthenogenesis in autotetraploid plants other than maize. It is known to occur in the autotetraploid *Oenothera gigas,* in *Datura* and in *Cucurbita* (Shifriss, unpublished). In *Euchlaena perennis,* a natural autotetraploid, there is evidence that parthenogenesis occurs very rarely. However, parthenogenesis has been reported in many diploids and allopolyploids, and since the phenomenon is fundamentally the same here as in autopolyploids it must presumably be reckoned with as a complicating factor in the development of doubled strains of horticultural and agronomic crop plants.

Under field conditions, the possible admixture of new autotetraploid strains with the parent diploid stock or other related diploid varieties must be considered. This would, of course, be a problem only with naturally cross-pollinated species. What has just been said about the crossability of spontaneous mutant diploids arising parthenogenetically in populations of autotetraploids applies

equally well to populations of diploids grown in proximity to related autopolyploids. There will be some intercrossing, but because of the relatively high cross-incompatibility of different members of autopolyploid series relatively little viable seed will be produced. The crossed seed normally will produce triploids which are themselves relatively infertile. However, seed set may be reduced appreciably, especially in the tetraploid, by interference of the pollen of the diploid with the normal fertilization of the tetraploid with its own pollen. At least, this is true in maize.

In this survey of induced polyploidy, an attempt has been made to evaluate the characteristics of autopolyploids in relation to their possible utilization in the production of superior varieties of cultivated plants. It is perhaps premature to venture a definite opinion as to the probable value of induced polyploidy as a method of plant breeding. But it is clearly apparent from the evidence at hand that the new strains of cultivated plants that are being produced by chromosome doubling furnish the plant breeder with a wealth of new material differing significantly in many important respects from the varieties at hand. These new tetraploid strains are most certainly worthy of very careful study, both by the geneticist and the plant breeder.

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## DISCUSSION

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DR. L. F. RANDOLPH[1] has suggested that "at the present time methods are available for producing tetraploid strains of most any plant." He has reviewed some of the results obtained. However, it is impossible in one morning to fully review all the promising suggestions for research which the various methods of inducing polyploidy now offer. These methods should not be considered merely as ways of originating a list of new improved varieties; rather such methods present the possibility of re-studying the origin, evolution and relationships of crop plants in a practical way. Though an end result of

[1] This is a brief discussion of a paper presented by Dr. Randolph at the joint meeting of the Genetics Society of America, the Botanical Society of America and the American Society for Horticultural Science at Philadelphia, Pa., Jan. 1, 1941.

such a re-study may be greatly improved varieties, the genic constitution and chemical composition of the various crop plants are so diverse that only the careful investigation of each can determine the full usefulness of polyploids and the approach to practical methods of improvement in any crop plant.

The necessity of having a cytologist within the breeding unit should also be pointed out. In some herbaceous crop plants induced polyploids may perhaps be immediately recognized by their gross morphological characteristics, but this is not the case with most polyploids of small fruits. Merely inducing polyploidy is not, then, a practical help in breeding berries except where cytological help is available.

In crops where polyploids occur naturally and where hybrids are easily made, as in the small fruits, doubling the chromosome number of the hybrids to obtain fully fertile seedlings may prove of very great value—of much more value than where the chromosome number of any given variety is doubled. This is so because such fully fertile plants may have highly desirable qualities from both parents as is the case in raspberry-blackberry hybrids, such as the Nessberry.

If the interpretation of Crane and Thomas in England is correct, the Logan (Loganberry) is a hexaploid resulting from the fertilization of an octoploid blackberry with a diploid (unreduced) pollen grain of a red raspberry. Such an interpretation suggests many questions. How often are unreduced pollen grains found? In what varieties and hybrids do they occur? Under what conditions are they produced in greatest quantity? Are diploid egg cells produced as often as diploid pollen grains? Can we separate out diploid pollen grains from haploids in a practical way? Having a polyploid blackberry series already (2n, 4n, 6n, 8n and 12n) we may be able to obtain $2n \times 2n$, $4n \times 4n$, $6n \times 4n$, $8n \times 4n$, and $12n \times 4n$ blackberry × raspberry hybrids in order to get improved Logan types. Crane and Thomas found a relationship

between the red raspberry chromosomes and one set of those in the Pacific Coast blackberry. This raises another question. Did the native Pacific Coast blackberry originate as a doubled hybrid between a hexaploid dewberry and a diploid black raspberry?

Crane and Thomas also obtained with polyploidy in Rubus changes in reproduction from sexual to apomictic and parthenogenetic reproduction. This suggests the potentialities of crossing widely different raspberries and blackberries.

The amount of experimentation necessary in any group may be indicated from the immense amount of material that can be used once the method of doubling is available. Native strawberries are diploid, hexaploid, and octoploid. Already through Dr. Dermen's work we have new triploid, tetraploid, pentaploid, hexaploid, octoploid, decaploid, 12-ploid, 16-ploid, and possibly many other ploids, and combinations of these. We can readily get many more new forms of strawberries than we originally had and need not be limited to the established concept of the strawberry in our aims. There are several or many species of diploid, tetraploid, and hexaploid blueberries which are heat- and cold-, and water- and drought-resistant to a remarkable degree, and only facilities and the energy and vision of the workers need limit the useful work to be done.

# TIME REQUIRED FOR DROSOPHILA MALES TO EXHAUST THE SUPPLY OF MATURE SPERM

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IN various experiments with x-rays it has been found that similar treatments occasionally give results which deviate widely from the mean. In all these experiments males were treated and the effect on the presumably mature sperm was studied by allowing the males to remain with one set of females for five or six days. Since the work of Harris (1929), Hanson and Heys (1929), Timofeeff-Ressovsky (1930, 1931) and Shapiro (1931) indicates that under similar circumstances the sperm which was immature during the treatment does not begin to be used until about the fifteenth day, the six-day mating period employed by us seemed to offer ample safeguard. However, the evidence mentioned above was obtained from copulations which had not been controlled, and the possibility was not excluded that if the males mate repeatedly they may exhaust the supply of the mature sperm in a period even shorter than five or six days. Such a condition would easily account for the variability observed by us. Therefore an experiment was planned to determine how long the supply of mature sperm is available under different conditions when the number of copulations is controlled.

The authors wish to express their sincere thanks to Margaret Hoover Brooks and to Ruth Bate Eckardt for considerable assistance in the collection of the data here presented.

## METHODS AND MATERIAL

Six-day-old males previously unmated and five- to six-day-old virgin females, both belonging to the wild-type Swedish-b stock of *Drosophila melanogaster,* were used in these experiments. Flies were kept at 22 degrees Cen-

tigrade except during the controlled mating periods when they were kept at room temperature. The experiment was conducted during January and February and the temperature of the room fluctuated between 18 and 22 degrees. All males and females used in this experiment were etherized only once, namely, immediately after hatching. During the experiment flies were handled without anesthetization.

All copulations were observed. Immediately after completion of a copulation, the female was separated from the male and placed in a vial containing a glass slide covered with culture medium on which eggs might be deposited. Our regular culture medium of corn-meal, molasses and agar was used, and to this was added a small amount of powdered charcoal to facilitate the egg counting. The food was painted with a suspension of fresh yeast in water. Slides were changed daily so that each contained the eggs laid during a twenty-four-hour period. The eggs were counted immediately after the slides were taken from the vials. The number of unhatched eggs was determined about fifty hours after the first count, and the pupae and the adults were counted at the time of emergence of the flies.

## Experimental Results

The major portion of the experimental data here presented concerns the proportion of lethals among the sperm ejaculated in repeated copulations following irradiation. The males used were given an x-ray treatment of 3,000 r-units which induced a high percentage of dominant lethals in those sperms which were in the later stages of their development at the time of irradiation. A sudden drop in the percentage of dominant lethals becomes evident as soon as the sperms, which were in earlier stages of development at the time of treatment, begin to be used.

*Stage of development at which lethal effect occurs.* From the counts on eggs laid, eggs hatched, pupae and

flies, determinations can be made regarding death rates during the egg, larval and pupal stages. These determinations indicate that the alterations induced in the sperm by 3,000 r treatment kill the great majority of individuals in the embryonic stages and only a few in the larval stages. Practically all the alterations which are able to survive during larval development live also through the pupal stages, since there is no evidence of excessive death rate among the pupae as compared with the controls. Under the conditions of this experiment dominant lethals expressed themselves mostly in the embryonic stages and to a lesser degree in the larval stages. Table 1 summarizes the pertinent data. In order to simplify the presentation of the data and to reduce the length of tables we

TABLE 1

SUMMARY OF DATA SHOWING PER CENT. OF SURVIVALS IN EGG, LARVAL AND PUPAL STAGES FROM FERTILIZATIONS WITH TREATED (3,000 R-UNITS) AND UNTREATED MALES

| Series | Total eggs | Per cent. surviving | | |
|---|---|---|---|---|
| | | eggs | larvae | pupae |
| | | | Treated males | |
| A ..... | 9,024 | 22.6 ± 0.4 | 82.1 ± 0.9 | 96.7 ± 0.4 |
| B ..... | 3,539 | 22.7 ± 0.7 | 94.0 ± 0.8 | 98.3 ± 0.5 |
| C ..... | 2,135 | 18.4 ± 0.8 | 93.6 ± 1.2 | 99.2 ± 0.5 |
| D ..... | 1,222 | 17.0 ± 1.1 | 92.8 ± 1.8 | 99.0 ± 0.7 |
| E ..... | 1,928 | 16.5 ± 0.8 | 88.1 ± 1.8 | 99.3 ± 0.5 |
| F ..... | 1,424 | 64.5 ± 1.3 | 92.2 ± 0.9 | 99.5 ± 0.2 |
| G ..... | 1,757 | 25.1 ± 1.0 | 84.1 ± 1.7 | 99.5 ± 0.4 |
| H ..... | 2,032 | 20.0 ± 0.9 | 88.2 ± 1.6 | 99.2 ± 0.5 |
| | | | Controls | |
| I ..... | 1,527 | 87.8 ± 0.8 | 97.2 ± 0.5 | 99.2 ± 0.2 |
| J ..... | 805 | 86.2 ± 1.2 | 98.1 ± 0.5 | 99.9 ± 0.1 |
| K ..... | 1,638 | 82.4 ± 0.9 | 99.3 ± 0.2 | 99.7 ± 0.1 |
| L ..... | 304 | 94.1 ± 1.4 | 96.9 ± 1.0 | 100 |
| M ..... | 430 | 92.1 ± 1.3 | 98.5 ± 0.6 | 100 |
| N ..... | 1,417 | 84.5 ± 1.0 | 99.1 ± 0.3 | 99.9 ± 0.1 |

shall give only the counts of eggs laid and of adults emerged, and omit those dealing with the larvae and the pupae. However, complete data are available and will gladly be supplied to any one who can make use of them.

The conclusion that unhatched eggs are killed by dominant lethals is based on the assumption that the eggs laid by an impregnated female have been fertilized. This assumption would not be valid if copulation should stimulate a female to lay unfertilized eggs. To settle this

question 99 eggs were studied which had been laid by females following mating with males previously x-rayed with 5,000 r-units. These eggs were fixed, sectioned, stained by the Fuelgen technique and examined microscopically to determine if fertilization had occurred. In 87 of the 99 eggs syngamy had taken place, whereas in one egg there was evidence that fertilization had not occurred. In the remaining 11 cases the chromosomes were not clearly defined because of inadequacy of the Fuelgen preparations. However, the presence in these 11 eggs of cytoplasmic islands which normally surround the division figures in fertilized eggs suggested that fertilization had likewise been effected in these cases. The result suggests that only one of 99 eggs examined was not fertilized. This evidence indicates that sperm treated by x-ray lives, functions and fertilizes eggs, but that a certain proportion of embryos arising in such eggs fail to develop because of changes induced by the treatment. Therefore our conclusion seems justified that the unhatched eggs are killed by dominant lethals.

*Description of the experiment.* Virgin, wild-type Swedish-b males six days old were given an x-ray treatment of 3,000 r-units. They were separated into three groups; the first was mated immediately with Swedish-b females, the second was mated 12 days after the treatment, and the third 19 days. With each of these groups a number of matings were conducted (series A to H), which are described below. Copulations were observed in each case and males separated from the females immediately thereafter. A separate record was kept for every male and every female.

*Series A.* Immediately after the treatment each male was mated with a single female. Initially 41 males were used, but only 16 were continued throughout the experiment. Each of these received a number from 1 to 16. This number was retained when the same males were used in subsequent series, B, C, D and F. After the copulation with the first female was completed, that female

was removed from the vial and the male received another female. This procedure continued from 7 o'clock in the morning until 2 o'clock in the afternoon. During that time each of several males mated as many as five times. Mated females were placed separately in vials containing glass slides bearing yeasted food for egg laying. The slides were changed daily throughout the life of the females.

*Series B.* Sixteen males from series A were mated again six days later. Copulations proceeded from 7:00 A.M. until 5:00 P.M. Each of two males copulated nine times and each of two others ten times.

*Series C.* Males from series B were mated again the next day (seven days after the treatment) to test the constitution of the sperm formed overnight. It was thought that the repeated copulation of the previous day might have exhausted the supply of mature sperm. In this series each male was allowed to copulate two or three times.

*Series D.* Males from series C which were also used in series A and B were mated again 5 days after series C or 12 days after the treatment. Each male was allowed to copulate twice.

*Series E.* Twenty males treated together with those of series A but not previously mated were allowed to copulate with two females each 12 days after the treatment.

*Series F.* Males used in A, B, C and D were mated again 7 days after the D matings and 19 days after they were x-rayed. Each copulated with two females.

*Series G.* Males from E which were tested only once 12 days after x-raying were mated again 7 days after the first test and 19 days after raying.

*Series H.* The third group of x-rayed males was mated for the first time 19 days after raying. Each male copulated twice.

*Controls.* A set of experiments comparable to series A to H was made with untreated males to serve as con-

## TABLE 2

SERIES A, B, C, D AND F. DATA SHOWING THE PERCENTAGE OF ADULTS OBTAINED FROM EGGS LAID BY INDIVIDUAL FEMALES MATED WITH MALES WHOSE COPULATIONS WERE CONTROLLED. MATINGS WERE MADE: (A) ON THE DAY WHEN MALES WERE IRRADIATED: (B) **6th day**; (C) 7TH DAY; (D) **12th day**, AND (F) 19TH DAY AFTER IRRADIATION. (E = EGGS LAID, A = ADULTS).

| Copulation: | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | | 6 | | | 7 | | | 8 | | | 9 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Male number | E | A | % | E | A | % | E | A | % | E | A | % | E | A | % | E | A | % | E | A | % | E | A | % | E | A | % |
| 1 .... | 74 | 12 | 16.2 | 62 | 19 | 30.6 | 112 | 32 | 28.6 | 63 | 16 | 25.4 | | | | | | | | | | 15 | 1 | | 6 | | |
| 2 .... | 55 | 8 | 14.5 | 80 | 17 | 21.3 | 140 | 39 | 27.9 | 100 | 31 | 31.0 | | | | 29 | 5 | 17.2 | | | | 11 | | | 59 | | |
| 3 .... | 73 | 12 | 16.4 | 58 | 14 | 24.1 | 14 | 1 | | 97 | 10 | 10.3 | 26 | | | | | | | | | 61 | 13 | 21.3 | 19 | 6 | |
| 5 .... | 74 | 10 | 13.5 | 103 | 26 | 25.2 | 97 | 19 | 19.6 | | | | 54 | 11 | 20.4 | | | | | | | | | | 55 | 7 | 12.7 |
| 6 .... | 64 | 10 | 15.6 | 75 | 20 | 26.7 | 60 | 10 | 16.7 | 44 | 4 | 9.1 | 119 | 29 | 24.4 | 88 | 12 | 13.6 | 74 | 16 | 21.6 | 45 | | | | | |
| 7 .... | 62 | 6 | 9.7 | 35 | 6 | 17.1 | 31 | 9 | 29.0 | 76 | 15 | 19.7 | 107 | 28 | 26.2 | 42 | 12 | 28.6 | 28 | | | 78 | 6 | 7.7 | 93 | 12 | 12.9 |
| 8 .... | 50 | 12 | 24.0 | 93 | 12 | 12.9 | 53 | 9 | 17.0 | 21 | 5 | | | | | 32 | | | 67 | 16 | 23.9 | 26 | 3 | 11.5 | 33 | | |
| 9 .... | 53 | 7 | 13.2 | 80 | 15 | 18.8 | 67 | 8 | 11.9 | 56 | 9 | 16.1 | 11 | 4 | | 94 | 24 | 25.5 | 74 | 16 | 21.6 | 57 | 15 | 26.3 | 19 | 2 | |
| 10 .... | 50 | 7 | 14.0 | 138 | 35 | 25.4 | 15 | 7 | | 17 | 1 | | | | | 54 | 6 | 11.1 | 95 | 20 | 21.1 | 42 | 12 | 28.6 | 24 | 5 | |
| 11 .... | 47 | 6 | 12.8 | 126 | 28 | 22.2 | 71 | 17 | 23.9 | 64 | 10 | 15.6 | 59 | 10 | 16.9 | 70 | 3 | 4.3 | 86 | 9 | 10.5 | 75 | 14 | 18.7 | 97 | 20 | 20.6 |
| 12 .... | 69 | 16 | 23.2 | 90 | 17 | 18.9 | 101 | 21 | 20.8 | 100 | 13 | 13.0 | | | | | | | | | | | | | | | |
| 13 .... | 58 | 12 | 20.7 | 53 | 6 | 11.3 | 84 | 11 | 13.1 | 33 | | | 87 | 27 | 31.0 | 75 | 14 | 18.7 | 97 | 18 | 18.6 | | | | 121 | 15 | 12.4 |
| 14 .... | 63 | 12 | 19.1 | 66 | 12 | 18.2 | 28 | | | 70 | 14 | 20.0 | 33 | 14 | 42.4 | 40 | 10 | 25.0 | 44 | 11 | 25.0 | 64 | 14 | 21.9 | 20 | 4 | |
| 15 .... | 69 | 14 | 20.3 | 82 | 19 | 23.2 | 55 | 14 | 25.5 | 93 | 12 | 12.9 | 119 | 30 | 25.2 | 52 | 11 | 21.2 | 95 | 13 | 13.7 | 36 | 4 | 11.1 | 60 | 9 | 15.0 |
| 16 .... | 71 | 15 | 21.1 | | | | 54 | 15 | 27.8 | 49 | 8 | 16.3 | 89 | 18 | 20.2 | 63 | 13 | 20.6 | 77 | 14 | 18.2 | 96 | 16 | 16.7 | 107 | 12 | 11.2 |

| Copulation: | 10 | | | 11 | | | 12 | | | 13 | | | 14 | | | 15 | | | 16 | | | 17 | | | 18 | | | 19 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Male number | E | A | % | E | A | % | E | A | % | E | A | % | E | A | % | E | A | % | E | A | % | E | A | % | E | A | % | E | A | % |
| 1 .... | | | | 93 | 17 | 18.3 | 52 | 3 | 5.8 | 94 | 2 | 2.1 | 83 | 12 | 14.5 | 29 | 5 | 17.2 | | | | | | | | | | | | |
| 2 .... | | | | 19 | | | 43 | | | 50 | 5 | 10 0 | 6 | 1 | | 56 | 4 | 7.1 | 76 | 8 | 10.5 | 45 | 3 | 6.7 | | | | | | |
| 3 .... | 39 | | | 85 | 18 | 21.2 | 83 | 11 | 13.3 | 62 | 5 | 8.1 | 46 | 6 | 13.0 | 145 | 107 | 73.8 | 68 | 41 | 60.3 | | | | | | | | | |
| 5 .... | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6 .... | 64 | 10 | 15.6 | 82 | 8 | 9.8 | 113 | 14 | 12.4 | 32 | 1 | 3.1 | 57 | 43 | 75.4 | 124 | 86 | 69.4 | | | | | | | | | | | | |
| 7 .... | 62 | 6 | 9.7 | 28 | 4 | 14.3 | 76 | 53 | 69.7 | | | | | | | | | | | | | | | | | | | | | |
| 8 .... | 11 | 1 | | 39 | | | 31 | | | 42 | | | 48 | 10 | 20.8 | 76 | 17 | 22.4 | 35 | | | 56 | 6 | 10.7 | 22 | | | 126 | 48 | 38.1 |
| 9 .... | 40 | 8 | 20.0 | 88 | 8 | 9.1 | 20 | | | | | | 178 | 100 | 56.2 | | | | | | | | | | | | | | | |
| 10 .... | 31 | 5 | 16.1 | 34 | | | | | | 27 | | | 10 | 1 | | 32 | | | 34 | | | | | | | | | | | |
| 11 .... | 15 | 12 | | 70 | 59 | 84.3 | | | | | | | | | | | | | | | | | | | | | | | | |
| 12 .... | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 13 .... | | | | 69 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 14 .... | 35 | | | 52 | 9 | 17.3 | 47 | 1 | 2.1 | 47 | 12 | 25.5 | 43 | 5 | 11.6 | 113 | 18 | 15.9 | 93 | 68 | 73.1 | | | | | | | | | |
| 15 .... | 114 | 95 | 83.3 | 40 | 27 | 67.5 | | | | | | | | | | | | | | | | | | | | | | | | |
| 16 .... | | | | 29 | 2 | 6.9 | 126 | 14 | 11.1 | 54 | 33 | 61.1 | | | | | | | | | | | | | | | | | | |

trol. The proportion of unhatched eggs was essentially similar throughout this experiment, indicating that the hatchability of eggs was not affected either by the age of the male or by the length of time the sperm was kept within the male. Summarized control data are given in Table 1.

## THE DATA

For all experiments the data covering the first 3 days of laying are added and given in the tables. The analysis of the complete data indicates that females mated once begin to lay an appreciable number of unfertilized eggs on the fifth or sixth days of laying.

Table 2 summarizes the data for A, B, C, D and F series in the order stated. The same set of males was used for all matings in these series. The males were mated immediately after raying for series A, on the sixth day for B, the seventh day for C, the twelfth day for D, and on the nineteenth day after irradiation for series F. In Table 2 the B and D series are printed in heavy type in order to distinguish them from the others. Vertical columns give the percentages of emerged adults

TABLE 3

SERIES E AND G. IN SERIES E MALES WERE MATED FOR THE FIRST TIME ON THE TWELFTH DAY AFTER X-RAYING, AND IN G THE SAME MALES WERE MATED SEVEN DAYS LATER, NAMELY, ON THE NINETEENTH DAY AFTER THE TREATMENT.

| | Series E | | | | | | Series G | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Copulation: | 1 | | | 2 | | | 3 | | | 4 | | |
| Male number | E | A | % | E | A | % | E | A | % | E | A | % |
| 1 .... | 38 | 3 | 7.9 | 66 | 12 | 18.2 | 133 | 22 | 16.5 | 25 | 4 | 16.0 |
| 2 .... | 37 | 9 | 24.3 | 47 | 4 | 8.5 | | | | | | |
| 3 .... | 56 | 3 | 5.4 | 13 | 2 | | 69 | 12 | 17.4 | | | |
| 4 .... | 34 | 8 | 23.5 | 34 | | | 30 | 5 | 16.7 | 108 | 41 | 38.0 |
| 5 .... | 56 | 4 | 7.1 | 53 | 1 | 1.9 | | | | | | |
| 6 .... | 38 | 1 | 2.6 | 60 | 4 | 6.7 | 17 | 3 | | 2 | | |
| 7 .... | 87 | 13 | 14.9 | 27 | 2 | 7.4 | 55 | 18 | 32.7 | | | |
| 8 .... | 22 | 5 | | 28 | 7 | 25.0 | | | | | | |
| 9 .... | 50 | 6 | 12.0 | 50 | 6 | 12.0 | 18 | 3 | | 103 | 23 | 22.3 |
| 10 .... | 73 | 9 | 12.3 | 42 | 10 | 23.8 | 141 | 19 | 13.5 | 78 | 21 | 26.9 |
| 11 .... | 85 | 18 | 21.2 | 47 | 10 | 21.3 | 125 | 12 | 9.6 | 123 | 33 | 26.8 |
| 12 .... | 69 | 4 | 5.8 | 31 | 6 | 19.4 | 28 | 1 | 3.6 | 36 | 6 | 16.7 |
| 13 .... | 42 | 4 | 9.5 | 77 | 7 | 9.1 | 48 | 12 | 25.0 | 36 | 13 | 36.1 |
| 14 .... | 44 | 8 | 18.2 | 61 | 12 | 19.7 | 115 | 15 | 13.0 | 88 | 14 | 15.9 |
| 15 .... | 85 | 14 | 16.5 | 69 | 15 | 21.7 | 78 | 8 | 10.3 | 66 | 8 | 12.1 |
| 16 .... | 75 | 16 | 21.3 | 43 | 6 | 14.0 | | | | | | |
| 17 .... | 82 | 18 | 22.0 | 70 | 15 | 21.4 | 22 | 5 | | 122 | 48 | 39.3 |
| 18 .... | 50 | 11 | 22.0 | 31 | 4 | 12.9 | 4 | 1 | | | | |
| 19 .... | | | | 49 | 4 | 8.2 | 35 | 8 | 22.9 | | | |

from successive copulations. All copulations are recorded, even though in some cases females did not lay eggs, or laid unfertilized eggs.

Table 3 summarizes the data for series E and G in which males were mated for the first time the twelfth day after irradiation and again on the nineteenth day. In Table 4 the data for series H are given in which males were mated for the first time on the nineteenth day after the x-raying.

TABLE 4

SERIES H. MALES WERE MATED FOR THE FIRST TIME ON THE 19TH DAY AFTER X-RAYING.

| Copulation: | 1 | | | 2 | | |
|---|---|---|---|---|---|---|
| Male number | E | A | % | E | A | % |
| 1 ..... | 47 | 7 | 14.9 | 19 | 2 | |
| 2 ..... | 91 | 13 | 14.3 | 121 | 19 | 15.7 |
| 3 ..... | 137 | 19 | 13.9 | | | |
| 4 ..... | 50 | 14 | 28.0 | 39 | 4 | 10.3 |
| 5 ..... | 71 | 12 | 16.9 | 86 | 13 | 15.1 |
| 6 ..... | 51 | 9 | 17.6 | 89 | 3 | 3.4 |
| 7 ..... | 139 | 34 | 24.5 | 15 | 3 | |
| 9 ..... | 89 | 15 | 16.9 | 131 | 33 | 25.2 |
| 10 ..... | 117 | 15 | 12.8 | 29 | 6 | 20.7 |
| 11 ..... | 87 | 14 | 16.1 | 109 | 21 | 19.3 |
| 12 ..... | 107 | 30 | 28.0 | | | |
| 13 ..... | 82 | 13 | 15.9 | 53 | 15 | 28.3 |
| 14 ..... | 70 | 10 | 14.3 | 87 | 31 | 35.6 |

## DISCUSSION

These experiments were planned to determine the length of time after irradiation that males may be bred before they begin to utilize the sperm which was immature at the time of treatment. By immature we mean the sperm which was at such a stage of development at the time of treatment that it gives a smaller percentage of dominant lethals when used subsequently in copulation than does the sperm which we call mature.

The experiments described earlier showed that the sperm exposed to 5,000 r-units functions and fertilizes practically all eggs laid by females inseminated with such sperm. Therefore, we are justified in assuming that a similar condition existed in this experiment, at least in cases where the males copulated immediately after treatment. In these cases we can assume that all eggs have

been fertilized and that the death rate during the development, in excess of death rate in the controls, indicates the frequency of dominant lethals induced by the treatment. In some instances, after repeated copulations, a decrease was observed in the percentage of the adults emerging. We attribute this decrease to the deposition of unfertilized eggs which are laid by females when the supply of sperm available for fertilizations is low. Therefore the percentage of adults emerging appears to be determined by three factors, (1) the use of the sperm which was mature at the time of the treatment as indicated by the percentage of adults obtained from copulations effected immediately after the treatment, (2) the use of immature sperm which is indicated by the rise in that percentage, and (3) the scarcity of sperm which is indicated by a decrease in that percentage.

Let us now analyze the data shown in Table 2 where we have summarized those experiments in which the individual males were allowed to copulate repeatedly on several days during the nineteen following the treatment. By consulting the data of first copulations it is evident that there is a great deal of variability among the different items. The extreme variants are $9.7 \pm 3.8$ and $24.0 \pm 6.0$, and the difference between them ($14.3 \pm 7.1$) is larger than twice its standard error and thus significant. That variability is probably caused by an occasional laying of unfertilized batches of eggs by certain females. However, for the present purpose it is not necessary to go into the analysis of the causes of this variability. It is sufficient to keep it in mind in drawing conclusions that more weight should be placed on the trend shown in consecutive items than on the individual differences, except in cases where these differences are very large.

An examination of the data on copulations effected immediately after the treatment, shown in the first light-type columns in Table 2, indicates no appreciable change in the percentage of adults obtained from four consecutive copulations. Data on an additional 25 males, which

are not given here, are in agreement with this evidence. This indicates that the supply of sperm in five-day-old virgin males is not appreciably reduced by four successive copulations.

The results obtained from copulations carried on with the same set of males six days later are shown in the first group of heavy-type columns. They indicate that the supply of available sperm was sufficient for about four copulations. A striking feature of this series is the fact that in cases where males copulated more than four times an appreciable proportion of copulations did not give any fertile eggs. This might have been due either to failure by the male to ejaculate or to the small amount of sperm in the ejaculum. It seems probable that, in some cases at least, the lack of sperm was the cause. For example, male number 2 copulated ten times within ten hours and the last five copulations did not produce any offspring. Similarly, male number 1 copulated nine times, and the last six copulations produced only one fertile egg. This indicates that the supply of the sperm which is ready for use is exhausted after repeated copulations.

In series C the same set of males was mated again the following day. Results of these matings show that a supply of mature sperm was made available during the 14-hour interval which elapsed between matings. However, the data indicate that the quantity of sperm is smaller than on the preceding day, since in a number of instances no sperm was transferred during the third successive copulation.

In the D series in which the same males were mated five days after the C series, not more than two copulations per male were allowed, and the data are not sufficient to determine whether the supply of sperm was exhausted through these copulations, although the low percentages of adults observed in the majority of these copulations suggests that the supply of sperm was not adequate.

The first increase in the percentage of adults emerging

is evident in the F series, which represents the matings made on the nineteenth day after treatment. That increase undoubtedly indicates the beginning of the utilization of sperm which was less affected by the treatment than the sperm used by the same males in previous matings. Every one of the nine males tested shows the effect. The most interesting case in this series is male number 14. The first copulation with that male gave $15.9 \pm 3.4$ per cent. of adults, while the second copulation produced $73.1 \pm 4.6$ per cent. It is evident that the change from one kind of sperm to the other kind occurred here between the first and the second copulations, and moreover that this was an abrupt change.

The data given in Table 2 indicate that the sperm which was immature at the time of treatment is not ready for use twelve days later even though all available sperm may have been exhausted on at least two occasions (males 1 and 8). These data show also that repeated copulations do not induce immature sperm to mature precociously. For example, the male number 14 copulated 15 times during twelve days, 12 of these copulations producing offspring, but despite this the sperm which was immature when treated was not ready for use for the first copulation on the nineteenth day. At the same time males number 11 and 15 which copulated only nine times during twelve days had such sperm ready for the first copulation on the nineteenth day.

The data of series E and G given in Table 3 support the conclusions reached earlier. In series E the males were mated for the first time on the twelfth day after treatment, and in series G the same males were mated again on the nineteenth day. These data do not show any considerable increase in the percentage of adults in any copulation. However, it is probable that in the last copulations with males number 4 and 17 and possibly with males 9, 11 and 13 a mixture of the two kinds of sperm was used. This indicates that the old sperm has to be utilized before the new sperm becomes available for use. That conclu-

sion is also supported by the data of series H shown in Table 4. Here the males were mated for the first time on the nineteenth day after the treatment. Among 12 males which copulated twice, only male number 14 showed in the second copulation a slight increase in the per cent. of adults.

Our data indicate that the sperm immature at the time of the treatment is not available for ejaculation on the twelfth day, even though males have previously mated and have exhausted the available supply of sperm. These data show also that the sperm which was immature during the treatment is ready for use on the nineteenth day, provided the old sperm is used up. Therefore such sperm begins to be used between the twelfth and nineteenth days. This is in complete agreement with the evidence obtained by Harris (1929), Hanson and Heys (1929) and Timofeeff-Ressovsky (1930, 1931) who used sex-linked lethals in their tests. A summary of their data is given in Table 5. Likewise, data furnished by Shapiro (1931) show that the frequency of translocations in the sperm used 1 to 16 days after treatment was 8.5 per cent., while in the sperm used 16 to 34 days after treatment the percentage dropped to 0.8.

TABLE 5

PERCENTAGES OF SEX-LINKED LETHALS OBTAINED FROM SPERM EJACULATED DURING CERTAIN PERIODS AFTER THE TREATMENT.

| Harris | | Hanson and Heys | | Timofeeff-Ressovsky | |
|---|---|---|---|---|---|
| Mating period days | Per cent. lethals | Mating period days | Per cent. lethals | Mating period days | Per cent. lethals |
| 1–4 | 8.6 | 1–7 | 5.6 | 1–5 | 6.9 |
| 4–8 | 9.7 | 7–14 | 6.4 | 5–10 | 8.3 |
| 8–12 | 7.3 | 14–21 | 2.9 | 10–15 | 7.3 |
| 12–16 | 1.7 | 21–28 | 2.1 | 15–20 | 4.0 |
| 16–20 | 0.6 | Control | 0.076 | 20–25 | 3.1 |
| 20–24 | 0.8 | | | 25–30 | 1.8 |
| Control | 0.2 | | | Control | 0.0 |

The first indication of the drop in the percentage of lethals was observed during the 12–16-day interval by Harris, and during the 15–20 day interval by Timofeeff-Ressovsky. These results would place the change at the fifteenth or sixteenth day. However, it seems very likely

that males may vary in this regard, as is indicated by the behavior of the male number 14 in Table 2. In that male the less affected sperm became available for the first time during the second copulation on the nineteenth day, while in all other males that sperm was available for the first copulation on that day.

The obvious conclusion to be drawn from these data is that the number of sperms fully matured and available for immediate transfer is sufficiently limited so that they would become exhausted in a few consecutive matings. This conclusion is substantiated by the fact that a number of females deposited only unfertilized eggs after they had mated with males which had completed several successive copulations. As our data (Table 2) show, however, males which have exhausted their sperms on one day may have functional sperms ready on a succeeding day, and in the case of irradiated males these sperms show no significant difference in the proportion of dominant lethals. We may presume, therefore, that sperms made available following "exhaustion" are derived from the mature sperms which almost fill the testis of the adult fly of this species (Huettner, 1930) but which are not made available for ejaculation until release of the earlier formed cells. The appreciable drop in the percentage of sex-linked lethals occurring in sexually active males approximately 15 to 19 days after irradiation would then be attributable to the maturation of sperms which were in an earlier stage of development at the time of treatment.

## SUMMARY

Males treated with 3,000 r-units were repeatedly mated on the day of the treatment, on the sixth day, the seventh day, the twelfth day and the nineteenth day thereafter. A drop in the percentage of dominant lethals was not observed until the nineteenth day, indicating that the sperm which was immature at the time of the treatment does not become available until some time after 12 days. Our evidence indicates that individual males vary consider-

ably and suggests that the less affected sperm becomes available between 15 and 19 days after treatment, provided the sperm which was mature at the time of treatment has previously been used. When males copulated for the first time on the nineteenth day after the treatment two copulations did not exhaust the old sperm.

The data show that the fully matured sperm available for immediate transfer may become exhausted in a few consecutive matings.

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# REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

IN this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

**Man The Mechanical Misfit.** By G. H. ESTABROOKS. New York: The Macmillan Co., 1941: i–xi, 1–251. $2.50.

PROFESSOR ESTABROOKS, a psychologist with anthropological training, herein expresses and enlarges upon the idea, in a rather one-sided viewpoint, that the line of human evolution is unquestionably and irretrievably headed toward early extinction. *Homo sapiens,* he is convinced, was marvelously evolved for savage life but is quite unadapted for civilized existence.

The author's arguments run about as follows: Various skeletal, muscular and visceral structures are maladjusted to modern life and have been subject to increasing degeneration since natural selection was eliminated. Persons less well endowed physically and mentally are outbreeding those with finer physiques and better brains. Social forces will continue to block all efforts to enact eugenic measures or to put them into effective operation. The human brain (naturally emphasized in a psychologist's treatment) is much too highly evolved for man's own good and for his racial security. Further retrogression in many characters is inevitable. "Man's best friend, the doctor," leads the social agents who benefit the individual at the dire expense of the race.

It is of vital significance that these thoughts be emphasized; that a rational, objective view be presented of the future of mankind. The book therefore serves an important and useful purpose.

On the alibi of popularizing his writing the author has

rather consistently personified nature and has given an appearance of dogmatism. In both respects he has succeeded so well that a spirit of mysticism and dogma will doubtless be felt by many in reading such passages as:

> Nature worked with the sea anemone and its relatives for a long time, then decided that she could do much better. She tried more experiments. A few million years, and she evolved model number two, the flatworm.

Apologies for such statements, offered in Foreword and as afterthoughts through the discussions, seem ineffectual excuses for relying on the tools of the nonscientific mind. By repeating, in effect, "I don't say what I really mean" the author has weakened his argument and has failed to imbue the reader with the spirit of the scientific method.

The text is sprinkled with inaccuracies and misinterpretations, mainly of a biological nature. Though mostly inconsequential in the general thesis, these errors will be appreciated by the informed reader and will probably be considered by him as he evaluates the conclusions on the future of man. This is unfortunate, as the biologist is particularly in need of enlightenment in eugenics.

In compensation many of the viewpoints expressed in the book exhibit a fine scientific approach. The reduction of "survival of the fittest" to "survival of the survivors" is particularly wise.

In the author's estimate of man's place in the future there is, despite some superficial appearance to the contrary, little of mysticism or wishful thinking, but plenty of courage, clear vision and sound human biology—with a goodly measure of pessimism, almost of fatalism or defeatism, thrown in. Human race progress, as in resistance to certain diseases, is now and then acknowledged but not given full attention. Almost no mention is made of the elimination of weaklings who break early under the strain of civilization, or of the destruction of those who, by reason of low mentality, slow reactions or lack of reasonable caution, fall victims to the speed of the machine age. Actual accomplishments in race betterment are slighted, and potential progress in eugenics is scarcely

considered. The reviewer would have looked for rays of promise in the release of the human mind from the bonds of mysticism, in the more complete acceptance of the doctrine of evolution, in the deeper and more widespread understanding of eugenic problems, in the growing appreciation of man's unique opportunity of controlling his own destiny.

**The Comparative Physiology of Respiratory Mechanisms.** By AUGUST KROGH. University of Pennsylvania Press, Philadelphia, 1941: 1–vii, 1–172, figs. 1–84. $3.00.

TESTIFYING to the fact that international cooperation in science is not yet dead, this treatise by the renowned comparative physiologist Krogh was produced under difficult circumstances. It presents in amended form lectures given at Swarthmore College in 1939, and was seen through the press while great nations tore at one another's throats, and small countries, like the author's Denmark, were ruthlessly overrun. The thorough although condensed treatment of the comparative physiology of respiratory mechanisms reviews in summary not only the extensive researches of the author but also the studies of other leading investigators, in all lands.

The treatment is truly comparative, with much emphasis on ecological relations, adaptation and evolution. All groups of animals are considered. The three introductory chapters deal with "The Call for Oxygen," "The Access to Oxygen" and "The Forces Acting in the Transport of Oxygen (and $CO_2$) through Living Tissues." An illuminating section on "Respiration in Water" is followed by a discussion, of ecological as well as evolutionary significance, on "Emergency Respiration. The Transition to Air Breathing." "Respiration in Air" is then similarly treated, with a skillful combination of brevity, clarity and completeness. Chapters on "The Respiratory Functions of the Blood" and "Tracheal Respiration" round out the discussion.

The author has succeeded well in his stated purpose, "to give representative examples of the ways in which the

problems of respiration and especially of oxygen supply have been solved in the animal kingdom, and to arrange these examples into a kind of physiological system, built up on analogy of function.'' Always the true scientist, and an active, penetrating experimentalist, Krogh closes this splendid little book with a statement of three ''lines along which research is desirable and will probably be fruitful.''

**Patterns and Problems of Development.** By C. M. CHILD. University of Chicago Press, Chicago, Illinois, 1941: i–ix, 1–811, figs. 1–224. $8.00.

THIS is the *magnus opus* of one of the deepest and most original thinkers among the leading biologists of the day. It covers and expands the life-work of Professor Child and his school of active researchers, and orients this work with a vast literature on problems of development. In his characteristically independent way the author departs from the usual treatment of development as the process of growth and differentiation from the egg. He views ova and spermatozoa as perhaps the most highly specialized of all body cells, and hence regards ontogeny as too complicated a process on which to formulate, at the present time, sound views as to patterns and problems of development. The phenomena of budding and reconstitution are regarded as simpler processes, more subject to critical experiments. Consequently such phases of development are stressed, with Child's concepts of the axial gradient and of the metabolic control of development as central themes.

There is no concise summary to clarify a long text that repeatedly passes through involved discussions, in which the reading tends to become heavy. However, the final chapter, ''Physiological Integration, Differentiation and Growth in the Progress of Development'' seems to be an integrating vehicle. In this chapter Professor Child shifts from an almost strictly physiological outlook to a more evolutionary point of view, stating:

. . . Development represents the reactions of a protoplasmic or cell system of a certain specific constitution to a spatial pattern. . . . The primary spatial pattern represents the primary ordering and integrating factor, but the character of the spatial and chronological order in development depends on the specific constitution. This is true not only for the whole organism but for particular organ systems. . . . We find, on the one hand, certain ordering or integrating factors, on the other, a capacity for independent differentiation or self differentiation of certain parts appearing at certain developmental stages in some forms. These two factors are, in some measure, mutually exclusive or antagonistic. . . . Growth is an important factor in development for morphological form, and proportions are largely results of differential growth.

. . . If gradient patterns are essential factors of development [as the author believes them to be], it is to be expected that genetic changes altering gradient patterns may often involve features apparently only slightly related or quite unrelated. The genetic change may alter whole gradient systems . . . and so alter localization, differential growth, and differentiation of many parts. . . . In so far as genetic changes permitting survival and reproduction have effects of this sort on the more general gradient patterns, organisms or organ systems evolve more or less as wholes with orderly relations of parts.

**A Biogeographical Study of the Ordinoides Artenkreis of Garter Snakes (Genus Thamnophis).** By HENRY S. FITCH. Univ. Calif. Publ. Zool., 44, 1940: 1–150, figs. 1–21, pls. 1–7. $1.50.

SNAKES again are the subject of an outstanding contribution to our knowledge of speciation. These animals seem to have a propensity for differentiation into seriated races, each of which tends to remain in its region of origin and to retain its characters with little change. The phyletic pattern thus tends to be diagrammatic. Fitch's study of certain garter snakes, like many other intensive researches on the plants and animals of the region, shows that such raciation is on a particularly fine pattern in the American west, where climatic and physiographic factors are sharply mosaic in arrangement. Within most of the subspecies as recognized the author finds unnamed races. Several of these local forms might better have been designated as subspecies too, but no reasonable nomenclatorial system would cover the minutiae of local races which this hard-working investigator has distinguished.

The most impressive feature of the study is the clear-

cut evidence that closely related links in the chains of local forms intergrade along narrow bands, and that only slightly less related forms within the species live together in complete genetic isolation. Thus *Thamnophis ordinoides elegans* intergrades regionally with three other subspecies, but in different parts of its range cohabitates with four other forms. Briefly, the primitive desert form (*vagrans*) is indicated as having given rise to two form-groups, one nicely adapted to terrestrial, the other to aquatic existence. The difference in behavior is particularly sharp where terrestrial and aquatic types occur together, and seem to avoid competition. Evidence for intergradation between the two ecological types could be found only for the most primitive subspecies in each line. Throughout the "artenkreis" regional intergradation is indicated as occurring between each derived form and its parent subspecies, except where climatic changes have introduced a barrier, but genetic isolation appears to have been achieved between each form and all grandparent, uncle and cousin subspecies.

Extensive field studies, with emphasis on distribution, habitat and food, coupled with a thorough analysis of new as well as old characters in about three thousand specimens, have given the author a penetrating insight into the snakes of the *Thamnophis ordinoides* group, and into their ecological and genetic interrelations. He has thus been able to find abundant and in general convincing evidence that "most of the geographic variation within the artenkreis appears to be adaptive," in opposition "to Ruthven's conclusion that evolution in *Thamnophis* has been along orthogenetic lines and nonadaptive." He even explains as secondarily adaptive the latitudinal tendency toward dwarfing, coupled with a reduction in number of scales. Other herpetologists, however, will find in Fitch's data strong support for the view of Ruthven, Blanchard and others that size of body and the correlated number of scales, scutes and vertebrae are both reduced peripherally from the center of distribution and presumed optimum range.

Climate, habitat, food supply and competition are very plausibly detailed as the factors which have controlled the evolution of these snakes.

**A Bibliography of Human Morphology 1914–1939.** By WILTON M. KROGMAN. Univ. Chicago Publ. Anthrop. (Phys. Anthrop. Ser.). Chicago: University of Chicago Press, 1941: i–xxxi, 1–385. $3.00.

IF THERE is a labor of love in science, it lies in the preparation of an inclusive bibliography. When the task has been thoroughly done, with references classified to subject by a leader in the field, the value of the service to fellow workers and to the science is grand. Professor Krogman has done such a job and deserves the praise and gratitude not only of physical anthropologists and all other vertebrate morphologists but also of workers in growth, mammalogy, general systematics, genetics, endocrinology, psychology, history and other sciences.

Titles are arranged by topic, as follows: Method in Physical Anthropology, Osteology, The Races of Man, The Prehistory of Man, Craniology, Teeth, Human Heredity, The Nervous System, Myology, Blood, Hair, Dermoglyphics (Hand and Foot), Studies in Phylogeny [including general references and citations to studies on other mammals as well as primates], Soft Parts, Body Type, Growth.

Much is told in the dedication of the book "To Dr. T. Wingate Todd who taught me that physical anthropology is more than bones and millimeters."

## NOTICES OF NEW BOOKS

The flood of new literature of interest to general biologists swamps any effort to review in detail more than a few selected items. Other books will be listed, with a few explanatory remarks, in the following fashion.

**The Ultracentrifuge.** By The Svedberg and Kai O. Pedersen. Oxford Univ. Press, 1940: i–x, 1–478, figs. 1–154. $11.50.—One of the International Series of Monographs on Physics, this book covers very authoritatively and in great detail an instrument that finds a main application in biological research.

**What Are the Vitamins?** By Walter H. Eddy. New York: Reinhold Publ. Corp., 1941: i–v, 1–247, 1 fig., 3 pls. $2.50.—We quote from the Preface of this comprehensive and well-documented book: "The story of the vitamins is today a long one, the literature enormous in volume. The present text is simply the author's personal effort to condense it without sacrifice of accuracy."

**The Control of Organisms.** By Frederick L. Fitzpatrick. New York: Bur. Publ., Teachers Coll., Columbia Univ., 1940: i–ix, 1–334, 50 figs. $2.75.—A well-written "story of efforts to dominate and control undesirable organic factors of the environment"—fairly comprehensive except in that the treatment, as in many ecological and wildlife works, stops at the water surface.

**A Manual of Aquatic Plants.** By Norman C. Fassett. New York and London: McGraw-Hill Book Co., 1940: i–vii, 1–382, many figs. $5.00.—This excellent manual, covering northeastern United States, is of particular value to ecologists and wildlife workers. It provides for the identification of sterile as well as flowering or fruiting plants.

**The Plant World A Text in College Botany.** By Harry J. Fuller. New York: Henry Holt & Co., 1941: i–xi, 1–592, figs. 1–306, 2 col. pls. $3.25.—An extremely attractive text, profusely illustrated with original photographs and drawings, and well designed to provide a thorough foundation in botany.

**Practical Zoology Instructions for Dissection and Preparation of Elementary Types of Animals.** London: Hutchinson's Scientific and Technical Publications (distributed by Chemical Publishing Co., New York), 1940: 1–118, 1 fig. and 6 diags. $2.00. —"Notes on the procedure and technique in the zoological laboratory."

**About Spiders Introducing Arachne.** By Elaine V. Emans. New York: E. P. Dutton & Co., 1940: 1–183, 22 figs., 9 pls. $2.50. —A charmingly written account of spiders, propagandizing the study of these outstandingly interesting animals.

## EDITORIAL

In this issue of The American Naturalist for the first time in many years there are 112 pages. This is sixteen pages over the usual ninety-six pages and attests to the favorable reception The American Naturalist has enjoyed, especially during the last two years.

An effort has been made to increase the subscription list to offset foreign discontinuances. The results are most encouraging and have made possible larger issues. We urge all those interested in the journal to assist in obtaining additional subscriptions so that more of the outstanding papers which are submitted for publication can be printed. If interest continues to increase, the journal may become a monthly publication such as it was over twenty years ago.

The American Society of Naturalists is appointing an editorial committee to assist in the selection of manuscripts. When it has been appointed the names will be announced.

With the continued support of biologists there is no reason why the journal should not enjoy a large circulation, and take its place as a link to bridge the gap between diversified biological subjects.

The journal will continue to improve and expand as long as it is needed and supported by those who read and write biology.

Jaques Cattell

# SHORTER ARTICLES AND DISCUSSION

## NOTE ON THE SEX RATIO AND MORTALITY IN TURKEYS

THE primary sex ratio is difficult to determine; hence most of the data for birds and mammals deal with the secondary or tertiary sex ratio. In some species of mammals there is evidence for an excess of males at the time of fertilization (Crew, 1937; Craft, 1938), while in birds McIlhenny (1940) has found that the primary sex ratios diverged from equality of males and females and that the secondary sex ratios did not differ greatly from the primary sex ratios of 30.3 per cent. males to 69.6 per cent. females in the boat-tailed grackle and 76.9 per cent. males to 23.3 per cent. females in the Gulf Coast redwing. Wide deviations from equal numbers of the sexes have also been reported for the tertiary sex ratios of birds by Mayr (1939), but such data furnish little or no information about the primary sex ratio, which is unknown for most species. Even for many species where some data are available, they are often, as pointed out by Henning (1939), too few or insufficiently critical to permit final conclusions.

The primary sex ratio of gallinaceous birds is unknown, but the secondary sex ratio tends to approach equality in the numbers of males and females. Certain species crosses result in wide deviations in the secondary sex ratio although, as shown by Sandnes and Landauer (1938), this depends upon the way the cross is made since reciprocal crosses between chickens and pheasants give different results. Where there is an equal number of males and females at the time of fertilization, this equality of sexes may be disturbed because of differential mortality. Landauer and Landauer (1931), MacArthur and Baillie (1932) and Crew (1938) have presented evidence which they consider favorably to the view that male birds suffer heavier mortality than females and that sex-linked genes have little influence on the secondary sex ratio in birds. On the other hand, Byerly and Jull (1935) have found greater female embryonic mortality among chickens and suggest that this is the result of impaired vigor caused by sex-linked genes. The data to be presented for turkeys and for chickens have a bearing on this problem.

### THE SEX RATIO IN TURKEYS

Data for turkeys for the years 1933–1939 are summarized in Table 1 together with data for chickens collected from 1935 to

1939 inclusive. The latter serve as a check series for comparison with published data for chickens and the data for turkeys.

TABLE 1

SUMMARY OF DATA ON THE SEX OF TURKEYS AND CHICKENS

| | Turkeys | | | Chickens | | |
|---|---|---|---|---|---|---|
| | Total no. | Males | | Total no. | Males | |
| | | No. | Per cent. | | No. | Per cent. |
| Dead germs* ..... | 515 | 285 | 55.34 | 326 | 184 | 56.44 |
| Failed to hatch+ ..... | 6,867 | 3,537 | 51.51 | 1,769 | 848 | 47.94 |
| Hatched—not banded.. | 755 | 397 | 52.58 | 399 | 217 | 54.39 |
| Hatched—banded ..... | 11,309 | 5,538 | 48.96 | 5,191 | 2,548 | 49.08 |
| Not allowed to hatch‡.. | | | | 861 | 437 | 50.75 |
| **Totals** ..... | **19,446** | **9,757** | **50.17** | **8,546** | **4,234** | **49.54** |

* Removed 5 to 7 days prior to the time of hatching.
+ Includes embryos that died during the last five to seven days in the incubator.
‡ Eggs removed at 18 to 20 days for experimental purposes.

The data for turkeys comprise all observations made on embryos and hatched birds (mostly of the Bronze variety) from nearly all eggs set in 1934 to 1939, inclusive. The turkey data for 1933 were for hatched poults out of families which were kept. Culling and disposal of entire families were based on egg production and hatchability. In 1936 data for 1,030 Bronze embryos from different breeders were included. These were supplied by the Donsing Breeding Farm and Hatchery, Rio Linda, and were out of eggs that failed to hatch. The chicken eggs were incubated and reared under comparable conditions and at about the same time of year as the turkeys.

The percentage of males based on all the data for turkeys does not differ significantly from 50 per cent. There was, however, a larger number (not statistically significant) of males among the embryos that died before the 20th day and among those that failed to hatch or died after the 20th day of incubation. If only the birds banded are considered, the percentage of males is less than 50 per cent. However, the percentage of males of all poults hatched was 49.20 per cent., and the deviation in this case is less than twice the standard error, whereas the excess of males amounting to 1.77 per cent. among all embryos failing to hatch is more than three times the standard error of the difference and hence significant. The difference in the sex ratio of the embryos and hatched poults of $2.57 \pm 0.74$ is also statistically significant. The excess of males among the unbanded hatched poults—most of which were crippled or weak so that they had to be discarded—is not statistically significant. Postnatal mortality for the four

years, 1935 to 1938, was 48.68 per cent. males out of 643 that died to 8 weeks of age and 63.29 per cent. males out of 158 that died from 8 to 16 weeks of age. These figures show an excess of males dying during the embryonic period with no significant difference for a few weeks after hatching followed by an apparently greater mortality among males.

The data for chickens (Table 1) agree, in general, with the published data for this species, the number of males being 49.54 per cent. at the earliest age to which they can be applied, which would be under 14 days of incubation. There was a larger number of males in the small sample of early dead in agreement with the data for turkeys, but as in the case of the turkeys the deviation was not statistically significant. After the 14th day of incubation, more female than male embryos died. There were again more crippled and weak (hatched—not banded) males than females. Of 553 chicks that died to 12 weeks of age, 52.26 per cent. were males. The excess of males is of doubtful significance, but agrees with the results of Landauer and Landauer (1931) and Dudley and Hindhaugh (1939) in showing greater postnatal mortality among males. The proportion of males was, therefore, under 50 per cent. at all ages for which data are available. No seasonal trends were observed in the sex ratios of either the chickens or the turkeys.

Sex was not determined for many embryos under 14 days of age and also not determined for a few older embryos, poults and chicks. The errors from this and other causes are believed to be small. The data for chickens agree with comparable data summarized by Byerly and Jull (1935). Data from commercial hatcheries reported by Crew (1938) are probably subject to larger errors of observation which may at least partly account for the higher percentage of males in the chicks. However, the embryos reported by Crew (1938) also show a higher percentage of males, although errors, if any, should be comparable to those in other published data. If the data presented in this paper, the data for embryos of Crew (1938), the data for hatched chicks of Dudley and Hindhaugh (1939) and the data of Byerly and Jull (1935) plus the earlier data summarized by them are combined, there are available records for 35,513 chicken embryos, of which $48.70 \pm 0.27$ were males, and 114,536 chicks, of which $49.35 \pm 0.15$ were males. Stated differently, the sex ratio for 150,049 chicks and embryos is $49.19 \pm 0.13$ per cent. males which in-

creased slightly at the time of hatch to 49.35 because of a higher mortality among female embryos during the last week of incubation. In each case the percentage of males is significantly less than 50 per cent. The proportion of male chickens (49.19 per cent.) is also presumably significantly lower than the proportion of male turkeys (50.17 per cent.), although the difference of 0.98 $\pm$ 0.38 per cent. is not quite three times the standard error. It should be kept in mind that there are significant differences in the sex ratios of the various chicken populations and of different breeds kept under comparable conditions (Byerly and Jull, 1935, and others).

## DISCUSSION

Several suggestions have been made to account for the deviation of the sex ratio in birds and other animals from equality of males and females. Those usually considered important, (Crew, 1937) include (1) sex-linked genes, (2) differences in the functioning of the male and female organisms, as exemplified by rate of metabolism, and (3) sex differences in response to environmental (including nutritional) factors. Chondrodystrophy, which in chickens affects male embryos more frequently than female embryos, is caused by manganese deficiency and is, therefore, an example of a sex difference in the response to a specific nutritional deficiency. Generalization from this single instance is not warranted, however, since in most cases more female than male chicken embryos die during the last week of incubation.

To postulate that the same factors operate with equal force in all populations seems unnecessary. It is much more likely that there are species and even strain differences in the number and importance of sex-linked genes, in the functioning and in the response to environmental factors of males and females. This would agree with the conclusion arrived at by Craft (1938) for interspecific and intergeneric mammalian hybrids. Data for chickens indicate that sex-linked lethal genes may have some influence on the sex ratio of at least some strains and breeds, whereas in the turkeys here studied this does not appear to be true.

## SUMMARY

Observations were made on 19,446 turkey embryos and poults, of which 50.17 per cent. were males. The sex ratio of hatched poults was 49.20 per cent. males, due to heavier mortality among male embryos.

The sex ratio of 8,548 chicken embryos and chicks was 49.54 per cent. males. When combined with comparable published data, the sex ratio obtained was 49.19 per cent. males or $0.98 \pm 0.38$ per cent. lower than for turkeys.

During the last week of incubation more male than female turkeys died, whereas the reverse was true for chickens. There was a larger number of males than females in small samples of embryos of both species that died between the time when sex could be readily identified and the last week of incubation, but the significance of this finding can not be established in absence of further data.

It is suggested that sex-linked genes have little or no influence on the sex ratio of the strains of turkeys studied here, whereas they perhaps exert an influence on the secondary sex ratio of at least some strains and breeds of chickens.

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# STERILE AMPHIDIPLOIDS: THEIR POSSIBLE RELATION TO THE ORIGIN OF *NICOTIANA TABACUM**

## INTRODUCTION

THAT amphidiploids which have complete and regular pairing of the chromosomes at meiosis always give rise to functional gametes and are fully fertile are assumptions generally accepted by geneticists. That these assumptions are unwarranted and that the fertility of amphidiploids is a matter of experimental determination and not one of prediction will be seen from the study of the four herein described.

Since the summer of 1937 the writer has made several amphidiploids to test the hypothesis that *Nicotiana tabacum* is of amphidiploid origin involving *N. sylvestris* and some one of the several species belonging to the *N. tomentosa* group; and, if possible, to reduce the number of possible ancestors of this group to the most likely ones. This hypothesis was first proposed by T. H. Goodspeed and R. E. Clausen (1928). It was based partly on the pairing relations of the chromosomes in the $F_1$ hybrids *N. tabacum* × *N. sylvestris*, *N. tabacum* × *N. tomentosa*, and *N. sylvestris* × *N. tomentosa*, and in *N. tabacum* haploid, and partly on the morphological features of the $F_1$ hybrids.

The writer has obtained three amphidiploids any of which may represent possible ancestors of *N. tabacum*. They are: *N. sylvestris-tomentosa*, *N. sylvestris-tomentosiformis* and *N. sylvestris-Setchellii*. The species *N. tomentosa*, *N. tomentosiformis* and *N. Setchellii*[1] are the most widely differing species of the *N. tomentosa* group, known to give Drosera scheme with *N. tabacum*, which were available to the writer. They exhibit striking differences in morphology, size and color of the flower as well as in the color and form of the vegetative parts. These three amphidiploids are readily obtained from callus tissue of decapitated stems which have been treated with a lanolin paste containing one per cent. indole-3-acetic acid (hetero-auxin) (Greenleaf, 1938).

The two amphidiploids *N. sylvestris-tomentosa* and *N. sylvestris-tomentosiformis* have been closely observed during two seasons and found to be completely female sterile. Meiosis is quite

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[1] A new species of *Nicotiana* collected by Mr. Bayne Beauchamp near Chachapoyas, Depto. Amazonas, Peru, in 1937, named and to be described by T. H. Goodspeed in University of California Publications in Botany, Vol. 18, No. 8, 1941.

regular and their pollen is 90 per cent. or more good. The parent species *N. sylvestris, N. tomentosa* and *N. tomentosiformis* have been grown and observed for many years by R. E. Clausen and myself. They have always proved to be fertile.

The third amphidiploid, *N. sylvestris-Setchellii,* is of considerable interest, as is evinced by the study of its female cytological behavior. This study shows that the plant is almost completely female sterile. The plant of *N. Setchellii* used in making the amphidiploid was self-fertile.

A fourth, completely sterile amphidiploid which is probably not concerned in the origin of *N. tabacum, N. glutinosa-tomentosa,* is also described. This amphidiploid, too, is a callus product like those which are described above. The $F_1$ *N. glutinosa* × *N. tomentosa* does not form callus shoots readily and the amphidiploid is, therefore, obtained only with considerable effort.

The results of extensive cytological studies of megasporogenesis and embryo sac development in the four sterile amphidiploids will be briefly set forth below. For comparative material of the normal type of behavior *N. tabacum purpurea* has been used. Unless otherwise stated, *N. tabacum purpurea* has also been used in the various crosses in this study.

### The Amphidiploids N. sylvestris-tomentosa and N. sylvestris-tomentosiformis

The amphidiploids *N. sylvestris-tomentosa* and *N. sylvestris-tomentosiformis* have quite regular meiosis. The homeotypic division of the chalazal nucleus is most often completed before that of the micropylar nucleus. Indeed, the latter sometimes disintegrates while still in metaphase or anaphase. The embryo sacs reach only the two or, at most, four nucleate stages when disintegration of the nuclei and collapse of the embryo sacs take place. By the time the flowers have fully opened many of the embryo sacs have already collapsed. Simultaneously with the disintegration of the embryo sac there usually occurs an enlargement of the integument cells which assume the place formerly occupied by the embryo sac. Two and four nucleate stages often persist for a very long time, even up to nine days or later after pollination; nevertheless, collapse and disintegration inevitably occur.

### The Amphidiploid N. glutinosa-tomentosa

The amphidiploid *N. glutinosa-tomentosa* also has fairly regular meiosis. Megasporogenesis is analogous to that of the two amphi-

diploids just described. No embryo sacs develop beyond the four nucleate condition. They all disintegrate in either the two or four nucleate stage. The development of the embryo sacs in this amphidiploid is very similar to that of the amphidiploid *N. sylvestris-tomentosa.*

### THE AMPHIDIPLOID N. SYLVESTRIS-SETCHELLII

The amphidiploid *N. sylvestris-Setchellii* occupies a position intermediate in embryo sac development between that of *N. tabacum* and that observed in the three sterile amphidiploids already described. Meiosis is fairly regular and embryo sac development is normal in some of the ovules. The majority of embryo sacs, however, disintegrate in the two or four nucleate stage, although some disintegrate in the eight nucleate stage. Fully formed eight nucleate embryo sacs occur now and then in the fully open flower. They have even been seen eight days after self-pollination. Pollen tubes fail to reach the fully developed embryo sacs in any number, if at all, even though an abundance of fresh pollen was always applied. Ovaries examined four, five, six, eight and nine days after self-pollination fail to show any pollen tubes in their apices. Styles of flowers examined three and four days after self-pollination and styles, four and five days after pollination with *N. tabacum* show only a few tubes near the base of the styles. The number of tubes increases as one approaches the stigma end of the style. Pollen tubes have been seen in the tip of only one ovary three days after self-pollination. Ninety-three self-pollinations and fourteen pollinations with *N. tabacum* have resulted in only one capsule with twenty "seeds" most of which apparently have no endosperm or embryo. Only two of the twenty "seeds" would appear capable of germinating. Almost all flowers dropped about ten days after pollination. This plant, nevertheless, gives some promise of being slightly fertile, perhaps when other pollen is used.

### THE STERILITY PROBLEM

A fertile amphidiploid *N. sylvestris-tomentosiformis* was reported by Kostoff (1938). It was made with the same strains of parent species as those employed by the writer in securing his corresponding sterile amphidiploid. Kostoff, over a period of several years, persistently crossed the extremely sterile $F_1$ hybrid *N. sylvestris* × *N. tomentosiformis* with *N. sylvestris.* He finally obtained a few seeds and among the resulting seedlings there were

two with thirty-six chromosomes. He then crossed these much more fertile, but still relatively sterile, plants with *N. tomentosiformis* and obtained a large number of seeds. Among the plants from this cross there were two with forty-eight chromosomes. These two plants are described by him as having been "almost fully fertile and were very much *N. tabacum*-like. Morphologically they were not quite alike. . . ." It must be added that their progeny also was not uniform, although a stable type was established after a few generations. Naturally this manner of obtaining the amphidiploid leads to chromosomal changes and it is not surprising that the two amphidiploids are not the same either morphologically or physiologically. The Kostoff plant differs greatly from the corresponding callus product both in the general appearance of the plant and in the morphology of the flower. Its habit is like that of *N. tabacum,* and unlike that of the callus amphidiploid. The callus method of making polyploids precludes chromosomal alterations. The chromosomal complement of the resulting amphidiploid is an exact duplication of that of the decapitated plant. It is thought that Kostoff's amphidiploid has undergone a quantitative alteration of chromosomal material with respect to the strictly duplicate condition present in the sterile amphidiploid. That this quantitative change has not been extensive is shown by the meiotic configurations in the $F_1$ hybrid *N. sylvestris-tomentosiformis* (4n) Kostoff × *N. sylvestris-tomentosiformis* (4n) Gr. The $F_2$ and backcross populations of this hybrid should throw light on the cause of the sterility. That the cause is nuclear, rather than cytoplasmic, is almost certain. To support this view the writer has made the reciprocal amphidiploid *N. tomentosiformis-sylvestris.* Although morphologically not identical with the amphidiploid *N. sylvestris-tomentosiformis* Gr—its flower is shorter and it has a broader limb—its embryo sac development reaches only the two nucleate stage when disintegration occurs.

The $F_1$ hybrid *N. sylvestris-tomentosiformis* (4n) Kostoff × *N. sylvestris-tomentosiformis* (4n) Gr, when superficially examined, appears to be quite fertile. Such, however, is not actually the case for on close examination the seed capsule, although containing hundreds of good seeds, contains a myriad more of aborted ovules. Extensive counts of uniformly large areas using a binocular microscope have been made fifteen days or later after pollination on pods from self-pollinations and crosses with *N. tabacum.* These counts show that only thirteen per cent. of the ovules de-

velop. Cytological examination of the aborting ovules shows clearly that the degeneration is similar to that in the sterile amphidiploids.

The same kind of cytological results as mentioned above have been obtained in the fertile $F_1$ hybrids *N. tabacum* × *sylvestris-tomentosa* (4n), *N. tabacum* × *N. sylvestris-tomentosiformis* (4n) Gr, and *N. tabacum* × *N. sylvestris-Setchellii* (4n). These three hybrids show respectively 17, 17 and 14 per cent. of developing ovules. In all these hybrids the disintegration occurs mostly in the two or four nucleate stage of embryo sac development. In all these hybrids with the exception of *N. tabacum* × *N. sylvestris-Setchellii* (4n) the per cent. of good pollen is high, ranging from 81 to 94 per cent. The evidence, therefore, points very strongly to a gene combination lethal only to the female gametophyte.

Further evidence favoring the view mentioned above comes from the $F_2$ (*N. tabacum* × *N. sylvestris-tomentosiformis* (4n) Gr) × S. Among seventy-three plants which have been observed sixty-eight are variously fertile, one with a few seeds per capsule, and two completely sterile plants. Two of the sterile plants of the $F_2$ population have been examined cytologically. In both embryo sac development is usually arrested in either the two or four nucleate stage, just as in the sterile amphidiploids previously described. An analysis of the cause of the sterility must, however, be confined to the use of the plants involved in the making of the sterile amphidiploids and not of *N. tabacum*.

## CONCLUSION

From the results reported it would be impossible to state exactly the origin of *N. tabacum*. Any one of the three amphidiploids reported is acceptable as a potential progenitor of *N. tabacum*. Taxonomically they all fall into the section *Tabacum*. Observations of chromosome pairing at meiosis in the p.m.c. of their hybrids with *N. tabacum* also support this conclusion. Recent evidence concerning other varieties of *N. tomentosa* shows that some of these give at least partially fertile amphidiploids with *N. sylvestris*. The importance which can be assigned to fertility as a criterion in deciding which species or varieties are involved in the ancestry of *N. tabacum* will depend on the complexity of the gene system responsible for the gametic abortion. If it is caused by only a few genes, it can not be assigned great importance, because these few differences may have arisen in the parental species since *N. tabacum* originated. If, on the other

hand, the fertile condition differs from the sterile one in many genes, then fertility as a criterion becomes of paramount importance. It is also quite possible that there may exist a still unknown and untried species or variety of the group which would give an amphidiploid more nearly representing the origin of *N. tabacum*. In any case the origin of *N. tabacum* can only be stated in terms of probability favoring some one particular combination, for the others can not be disregarded. Although fertility is an important criterion in reaching a decision, it is the aggregate of genes which the amphidiploid possesses in common with *N. tabacum* which is the real test for closeness of relationship. At present it is only possible to state definitely that *N. tabacum* is of amphidiploid origin and that its ancestors were similar to the species here employed.

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## PEARL IN QUOHOG

WELL-FORMED, large, lustrous pearls are often found in pearl oysters, *Pinna*, the abalone and many species of fresh-water mussels, but are very rarely encountered in quohogs (*Venus mercenaria*). This is, apparently, because in the quohog the edge of the mantle is quite firmly attached to the shell along its pallial line. Such condition excludes the possibility of a foreign particle gaining entrance into the space between the mantle and the shell, where it could serve as a nucleus for a pearl. Therefore, finding a good pearl in this animal is rather unusual and merits a description.

The pearl of rare size, shape and lustre and the quohog in which it was found were brought to the author by the clam-digger who found them. According to the clam-digger, the animal was dug from a mud flat in the Indian River, a tributary of Milford Harbor. The clam was at least 5 years old. Both shells showed a healed scar extending from the umbo to the edge (Fig. 1). The pearl was of a perfect oval shape 1.4 cm long, 1.1 cm

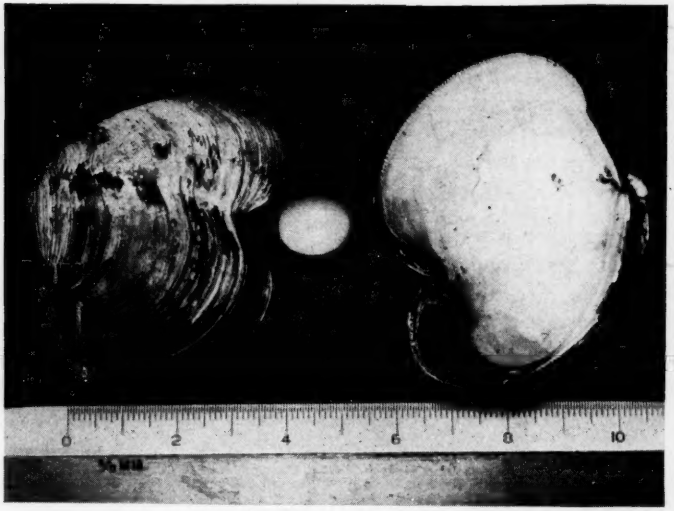


FIG. 1. Two shells of the quohog and the pearl they contained. Note the healed scar on the shell. The scale at the bottom of the picture is in centimeters.

wide and weighed 3.1 grams. Its color was lustrous milky-white with a delicate pink tint. Unfortunately, because of careless handling, this beautiful pearl had a crack on its surface.

It is thought that in this case the formation of the pearl was induced at the time the shell of the quohog was broken. Apparently a fragment of shell or grain of sand was lodged between the inner surface of the valve and the mantle, and remained there after the broken shell was healed.

I wish to express my thanks to Mr. J. B. Engle for taking the photograph of the pearl and the shells.

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